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A BOOK IN HIS HAND

I saw a man clothed with rags standing in a certain place, with his face from his own home, a book in his hand, and a great burden upon his back.

JOHN BUNYAN, "The Pilgrim's Progress" (1678).

To study the phenomena of disease without books is to sail an uncharted sea, while to study books without patients is not to go to sea at all.

SIR WILLIAM OSLER (1901).

WITH monosyllabic purity of diction, the opening sentences of *The Pilgrim's Progress* exemplify the brief perfection reached by the English language in the seventeenth century. In quite another sense they reflect our own situation. The doctor of today, like Christian, "a book in his hand", is increasingly dependent on the written word for professional enlightenment; good medical reporting is more essential than ever. The prime purpose of scientific writing, indeed of all language, is the accurate transfer of information from one mind to another, and to achieve this the words we use must have the same meaning for all who read. When we speak of a cup or a chair the risk of being misunderstood is minimal, but as we move from the concrete to the abstract, mental images conveyed by words tend to multiply. A speaker may use such a term as "socialism", and it can convey as many shades of meaning as there are listeners. The same phenomenon is not foreign to the professional scene, and something of this was in the mind of Sir George Pickering[1] when he stressed the proper use of words in clinical medicine. He showed that some, such as shock, arteriosclerosis, hypertension and bronchospasm, are interpreted quite differently by different doctors.

The language of medicine has two components, defined by a recent authority[2] as the technical terms, which are the bricks, and plain English words, the cement binding these. They merit separate consideration. Years ago Sir Clifford Allbutt deplored that medicine was becoming more an applied science than an art. The speed of change has accelerated, but nobody would wish to set back the clock of medical research with all its momentous application to the practice of our profession. Inevitably there results a compelling need to transmit ideas of greater complexity into fields more highly specialized than ever. Ffrangcon Roberts[2] puts it thus:

> As the various branches of medicine develop they create their own vocabularies which are understood only by those who habitually use them.

Hundreds of new words, which must carry a precise scientific meaning, are introduced each year, and the sound and sight of them ill accord with the spirit of the classical scholar. The term "agammaglobulinæmia" pleases nobody, but the physician cannot for ever

[1] *Brit. med. J.*, 1958, **2**, 1117.
[2] Ffrangcon Roberts, "Good English for Medical Writers", 1960, Heinemann.

refer to " the state in which the gamma globulin fraction of the serum is absent ". The age of technology is with us, and to it the language of the profession must conform. In so doing let us not forget that medical terminology has more than utility value and at times a cultural heritage of incisive accuracy. What could be more fitting than " atlas " for the first cervical vertebra, or " cancer " for the disease which Ægina so defined in the seventh century, because " it adheres to any parts which it seizes upon in an obstinate manner like a crab " ?

The quickening pace of medical advance has other implications. In this Journal, Professor Blackburn[3] has pointed out that physicians cannot intercommunicate professionally as a total group ; more and more are they stratified by age with its attendant distance from the basic sciences. In the face of this sad truth the need for good medical writing is accentuated. As the language of the special fields becomes more remote from ordinary speech, at least all should be able to comprehend the general purport of what is written.

The separation of scientific writing into technical terms and plain English words is in some sense artificial. As Sir Clifford Allbutt[4] has it in his " Notes on the Composition of Scientific Papers " :

> . . . though composition is painful, the man of science ought best to know that style and matter can no more be dissociated than skin and bone ; that if we write clumsily, loosely or disjointedly our thoughts also are undisciplined : for the sifting of language is the weighing of thought and in scientific prose words should be used as carefully as symbols in mathematics.

In the face of technical necessities medical writing of the day cannot be euphonious, and style must often be sacrificed to scientific integrity. Nevertheless good medical authors, and many of these we have, do achieve a presentation which can be read rapidly and with continuity. Choice of words and simplicity of diction are the keys, and success comes infrequently to the amateur in any degree pen-happy. Somerset Maugham has emphasized how exacting is the task of mastering the English language. If professional writers need years of training, how difficult for the embattled doctor to write well, in such time as he can spare to it.

In a statement on the preparation of papers in one of the more exclusive special journals, this telling epigram appears :

> " Easy writing's curst hard reading."—Richard Brinsley Sheridan.
> " Easy reading's curst hard writing." The Editors, *J. gen. Microbiol.*

Good composition requires revision and excision to the point of exhaustion, an ideal defined as the maximum of clarity in a minimum of words. That so much quality is revealed in our publications in the face of deep professional preoccupation is a matter for favourable comment. This is a point which can be taken further. Criticism of the present standard of medical writing is frequent. From outside it has been assailed by no less an authority than Sir Ernest Gowers[4] in his Oslerian Oration of 1958. Within the profession, there is pedantic criticism from medical scholars bred in the tradition of " classics in the morning, mathematics in the afternoon ". But the total scene is one of pace and change, and language marches with the times. The powerful but ponderous prose in which the giants of the past introduced their clinical concepts would hardly meet the needs of the records of today with their *staccato* biochemical crackle.

[3] AUST. ANN. MED., 1961, **10**, 1.
[4] Quoted by Sir Ernest Gowers, *Practitioner*, 1958, **181**, 338.

In a broader scene the combination of philosophic thought with the keen analytical medical mind has produced distinguished contributions to English literature. Of such is the Hunterian Oration entitled " The Commemoration of Great Men "[5] delivered by Wilfred Trotter in 1932 before the Royal College of Surgeons of England. Here " pearls of the mind are set in chosen words, but so set that they are to be seen by the mind rather than by the eye ". One idea of the orator has application to the present theme :

> The mind delights in a static environment, and . . there is any change to be itself the source of it. Change from without, interfering as it must with the sovereignty of the individual, seems in its very essence to be repulsive and an object of fear.

And again :

> The only way to the serene sanity which is the scientific mind—but how difficult consistently to follow—is to give to every fresh idea its one intense moment of cool but imaginative attention before venturing to mark it for rejection or suspense as alas nine times out of ten we must do

This outstanding address might well be compulsory post-graduate reading, and if reference to it here should induce but a single doctor to renew his association with the full text, then this presentation will be well justified. Nor do our colleagues in Australia and New Zealand neglect the call to write and speak the finest English when the occasion arises. Let those who disagree study editorials in these columns and in *The Medical Journal of Australia*, or the appreciations of our dead composed with such deep feeling, sincerity and insight. Or again, let them read such words as were spoken by a President of this College[6] to the graduating students he examined at Otago Medical School in 1952, or the address with which Dean Sayers[7] opened the Annual Meeting of the British Medical Association in Auckland earlier in this year. The art of expression with lucidity, force and simplicity is here in the face of all technical pressures ; the medical Christian still walks with " a book in his hand ".

J. O. MERCER.

[5] Trotter, W., *Brit. med. J.*, 1932, **1**, 317.
[6] McDonald, C. G., Graduation Address, *N.Z. med. J.*, 1953, **52**, 1.
[7] Sayers, E. G., *N.Z. med. J.*, 1961, **60**, 93.

RENAL ISCHÆMIA AND HYPERTENSION : A REVIEW OF THE RESULTS OF SURGERY[1]

PRISCILLA KINCAID-SMITH[2]
From the Baker Medical Research Institute, Melbourne[3]

SUMMARY

A review of the results of renal surgery in patients with hypertension reveals that 37% have a reduction in the blood pressure to 140/90 mm. Hg for a year. About half the patients achieve worthwhile clinical benefit from operation. The percentage of successful results in patients with renal artery disease or chronic pyelonephritis is almost double that achieved in other forms of unilateral renal disease. Patients aged under 20 years, and those with retinitis or papillœdema, seem to have a better prognosis for alleviation of hypertension than older patients or those with lesser retinal changes. When pyelonephritic scarring of the " ischæmic " type is present in the nephrectomy specimen, the operation is likely to alleviate hypertension. In patients with renal artery disease, nephrectomy produces a far higher percentage of successful results than is achieved by arterial surgery.

THERE has been renewed interest in the relationship between ischæmic renal parenchyma and hypertension following the widespread use of new diagnostic techniques such as aortography.

Numerous cases of stenosis of the renal artery have been recorded, and it is stated that, in over 80% of these, hypertension may be relieved by nephrectomy or arterial surgery (Thompson and Smithwick, 1952 ; Gellman, 1958 ; Morris *et alii*, 1960). In contrast, the last major review of the results of nephrectomy for hypertension revealed that in only 26% of 575 cases was there a reduction of the blood pressure to 140/90 mm. Hg for a year (Smith, 1956). This striking difference calls for an evaluation of the over-all results of nephrectomy and arterial surgery in the past few years. Three hundred and twenty-six cases recorded in the available literature since 1956 are therefore reviewed, and an attempt is made to assess the prognosis in terms of the underlying pathological process. Six new cases are included, and the histological features of these and other cases are discussed in relation to the causation of renal hypertension.

REPORTS OF CASES

CASE I.—A male patient, aged 32 years, had had headaches for one year. He had been known to have hypertension for six months, his blood pressure being 230/120 mm. Hg. Retinal hæmorrhages were present, but there was no papillœdema. Proteinuria was present (30 to 100 mg. per 100 ml.). The blood urea level was 21 mg. per 100 ml. Intravenous pyelography revealed good excretion and normal calyces on both sides. The right kidney was 12 cm. in length and the left kidney 14 cm. Aortography was unsatisfactory. Six months' treatment with hypotensive drugs controlled the blood pressure inadequately, but the retinitis resolved. Repeated aortography showed apparent stenosis of the right renal artery. Exploration revealed a long and possibly kinked right renal artery with a palpable thrill, but no obstruction within the lumen. Nephrectomy was performed because of the thrill and scarring of kidney. The blood pressure fell immediately to 120/70 mm. Hg and remained at about that level until the last observation 18 months after operation, when it was 125/80 mm. Hg.

Pathological examination of the kidney gave the following information. Its weight was 130 grammes (normal, 180 grammes). A scar measuring 3 by 2 cm. was present at the upper pole, and there were three smaller surface scars. Microscopic examination of the scarred areas showed partial infarction (ischæmic tubular atrophy) without inflammatory changes. Doubtful generalized ischæmic atrophy was observed. No block was demonstrated in the renal artery branches. There were benign hypertensive changes in the arterioles.

Apparent renal artery stenosis was thought to be present on the aortographic findings, and a thrill but no obstruction was demonstrated at operation, though there was possible kinking of the artery. A poor response to hypotensive drugs and visible kidney scarring justified nephrectomy. The pathological lesion was patchy partial infarction, which may occur with narrowing of a main renal artery.

CASE II.—A male patient, aged 54 years, presented himself with hypokalæmia and malignant hypertension and a small left kidney. The details have been recorded elsewhere (Dollery *et alii*, 1959). Nephrectomy produced an immediate fall in blood pressure to normal levels. A further follow-up examination since the previous report shows that the patient has remained

[1] Received on May 5, 1961.
[2] Sydney William Jones Medical Research Foundation Fellow.
[3] Postal address : c/- Alfred Hospital, Commercial Road, Prahran, S.1, Victoria.

normotensive for over a year; 18 months after operation the blood pressure was 130/85 mm. Hg. Pathological examination of the kidney revealed ischæmic renal atrophy due to athero-thrombotic occlusion of a major branch of the renal artery.

This patient presented with hypokalæmia; the serum potassium content was restored to normal by nephrectomy. The relationship between the hypokalæmia and ischæmic histological changes is discussed below.

CASE III.—A female patient, aged 51 years, had undergone an hysterectomy six years before, when her blood pressure was 160/100 mm. Hg. She presented herself complaining of recent headaches; her blood pressure was 230/120 mm. Hg. No retinitis or papillœdema was present. A trace of protein was detected in the urine; the blood urea level was normal. Intravenous pyelography revealed a small right kidney shadow with no excretion of contrast medium on that side. A ureteric catheter could not be passed up the right ureter, and it was assumed that it had been ligated during hysterectomy six years before. A right nephrectomy was performed and the blood pressure fell to 160/90 mm. Hg in the first week, and varied from 135/75 to 160/100 mm. Hg over the next two years. The kidney removed was small, and there was no evidence of hydronephrosis. Histological changes of partial infarction were seen. There were no vessel changes of hypertension.

The histological findings of ischæmic tubular atrophy were unexpected in view of the clinical assumption that the ureter had been tied. Since the renal artery was not explored, the ætiology of the renal lesion remains obscure.

CASE IV.—A male patient, aged four years, presented himself with facial paresis, restlessness and night sweats and a blood pressure of 260/150 mm. Hg. Papillœdema and heavy proteinuria were present. The blood urea level was 35 mg. per 100 ml. Intravenous pyelography showed that the right kidney length was 7·5 cm. and the left kidney length was 9 cm. Contrast medium was excreted well on both sides. Hypotensive treatment was ineffective. The parents failed to attend for 10 months, and then the child was readmitted to hospital with a blood pressure of 285/190 mm. Hg and hypertensive heart failure. Intravenous pyelography showed that the left kidney measured 8·5 cm.; the right was still 7·5 cm. long. Aortography revealed three small twigs to the right kidney and a normal left renal artery. The blood urea level was 44 mg. per 100 ml. Hypotensive drugs were ineffective. A right nephrectomy was performed, but the blood pressure did not fall and the blood urea level rose steadily to 815 mg. per 100 ml. at death a month later.

Pathological investigations gave the following results. Three vessels supplied the right kidney, which weighed 42 grammes. Most of the surface was dark, smooth and atrophic, but an area on the posterior surface supplied by the largest of the three renal arteries was raised and hypertrophied, with coarse surface nodularity due to the inter-lobular artery narrowing of malignant nephrosclerosis.

Histological examination of the raised area on the posterior surface showed interlobular artery narrowing with consequent adjacent areas of hypertrophied and atrophied renal parenchyma, and also abundant fibrinoid arteriolar necrosis. Histological examination of the relatively ischæmic areas supplied by the small renal arteries showed fibrinoid arteriolar necrosis without interlobular artery narrowing.

At autopsy, extensive interlobular artery narrowing with parenchymal ischæmia and fibrinoid arteriolar necrosis was found in the left kidney.

The interlobular narrowing with consequent ischæmic atrophy in the left kidney probably maintained the hypertension after operation and accounted for the rapid downhill course.

CASE V.—A male patient, aged 31 years, nine years previously had been found to have a hydronephrosis, which had been treated by ligation of an aberrant artery to the left lower pole. His blood pressure was 150/90 mm. Hg at that time. Immediately after operation the blood pressure rose to 190 mm. Hg systolic, and three months later it was 180/120 mm. Hg. Over the next five years the blood pressure readings varied between 140/90 and 160/80 mm. Hg. Nine years after operation the blood pressure was found to be persistently between 190/120 and 200/150 mm. Hg. Proteinuria was present. The fundi were normal. Intravenous pyelography showed that the left lower pole appeared contracted and contained a small calculus. The left kidney showed some hydronephrosis and measured 12 cm. The right kidney measured 14 cm. An aortogram showed an ischæmic wedge at the lower pole of the left kidney. At operation patchy surface scarring was found, together with a large scar at the lower pole. A left nephrectomy was performed. The blood pressure fell at once to 120/80 mm. Hg and remained normal for one week in hospital. Readings have varied between 140/90 and 145/105 mm. Hg over the subsequent twelve months.

The kidney weighed 140 grammes. There was moderate hypernephrosis, with an old fibrous infarct at the lower pole. Microscopic examination revealed parenchymal lesions of hydronephrosis and vessel changes of benign hypertension.

Although ligation of the aberrant renal artery produced an acute rise in blood pressure, it is not possible to assess how much it contributed to subsequent hypertension.

CASE VI.—A male patient, aged 45 years, complained of headaches. Hypertension was known to have been present for three years. His blood pressure was 220/130 mm. Hg. No retinitis or papillœdema was present. There was slight proteinuria, and the blood urea level was 40 mg. per 100 ml. Intravenous pyelography revealed a small kidney on the right. Contrast medium was excreted on both sides. Ureteric specimens showed a smaller volume but higher sodium content on the right side, suggesting that the renal lesion was not ischæmic and that nephrectomy would not alleviate the hypertension (Connor *et alii*, 1957). There was an unsatisfactory response to hypotensive drugs. The patient was readmitted to hospital five months later with persistent angina and hypertension. A right nephrectomy did not produce any significant change in the blood pressure. The patient died suddenly seven months later and no autopsy was obtained.

The kidney weighed 90 grammes, and pathological examination showed acute and chronic pyelonephritic lesions. The pyelonephritic scarring was mainly fibrous, without areas of ischæmic atrophy. Vessel changes of benigh hypertension were present.

TABLE I

Clinical Data on 326 Cases of Hypertension Treated by Renal Surgery since 1956

Author	No Significant Reduction of Blood Pressure	Improved Blood Pressure (but Not Reduced to 140/90 mm. Hg)	Blood Pressure 140/90 mm. Hg, but Less than One Year Follow-up	Blood Pressure 140/90 mm. Hg or Lower for One Year	Sex	Age (Years)	Operation*
Adams and Newman, 1958				+	M.	48	1
Aldman, 1956			+		M.	21	1
Amsler, 1958		+•			F.	47	1
		+			F.	24	1
	+				F.	44	1
		+•			F.	60	1
Birch-Jensen and Røjel, 1958	+				M.	49	1
Borhan and Lee, 1958	+				—	—	1
Brown *et alii*, 1960				+	M.	47	2
		+•			M.	45	1, 2
			+•		M.	47	2, 3
	+				M.	40	2, 4
	+				M.	52	1
	+				F.	49	4
		+			F.	40	1
		+			M.	28	1
			+		F.	21	3
				+	M.	39	1
Brust and Ferris, 1957				+	M.	28	1
				+	F.	38	1
				+	M.	4½	1
				+	F.	11	1
	+				—	—	1
	+				—	—	1
	+				—	—	1
	+				—	—	1
	+				—	—	1
	+				—	—	1
Castillo and Barrera, 1958		+•			M.	59	2
Connor *et alii*, 1957		+•			M.	39	1
		+•			M.	37	1
		+•			M.	50	1
		+•			M.	61	1
				+	F.	20	1
	+				M.	57	1
	+				F.	18	1
	+				F.	42	1
	+				F.	9	1
		+			F.	54	1
				+	F.	37	1
	+				M.	43	1
		+			F.	65	1
		+•			M.	68	1
				+	M.	66	1
				+	M.	61	1
		+•			F.	34	1
		+•			F.	52	1
	+				?	?	1
	+				?	?	1
	+				?	?	1
Connor *et alii*, 1960	+				F.	58	1
	+				M.	55	1
	+				F.	56	1
	+				F.	37	1
				+	M.	14	1
				+	M.	8½	1
				+	M.	40	1
			+•		F.	40	2
			+•		F.	27	1
Cordonnier, 1959		+•			M.	62	1
				+	M.	55	1
		+•			M.	53	1
			+•		F.	26	1
			+•		M.	54	1
		+•			M.	44	1
		+•			M.	52	1
Cottier *et alii*, 1958				+	M.	14	1
De Camp and Birchall, 1958				+	M.	9½	1
				+	F.	10	3
					M.	23	4
		+•			M.	69	1
		+			M.	64	1
Dix, 1957				+	F.	31	1
Dollery *et alii*, 1959		+•			M.	56	1
Douglas *et alii*, 1959		+•			F.	48	1
					M.	10	1
					M.	36	1
Dunn, 1958					M.	32	1
				+	M.	20	1
				+	M.	56	1
	+				—	—	1
Gellman, 1958				+	M.	65	1
Gilfillan *et alii*, 1956				+	M.	47	1
Glanton and Parsons, 1959				+	M.	47	1
Gollman, 1958			+		M.	18	1
Götzen, 1956		+•			M.	?	1

*1 = Nephrectomy. 2 = Thrombo-endarterectomy. 3 = Spleno-renal anastomosis. 4 = Other reconstructive arterial surgery. 5 = Segmental nephrectomy.

TABLE I—*Continued*

Clinical Data on 326 Cases of Hypertension Treated by Renal Surgery since 1956—Continued

Author	No Significant Reduction of Blood Pressure	Improved Blood Pressure (but Not Reduced to 140/90 mm. Hg)	Blood Pressure 140/90 mm. Hg, but Less than One Year Follow-up	Blood Pressure 140/90 mm. Hg or Lower for One Year	Sex	Age (Years)	Operation*
Haller *et alii*, 1957		+		+	F.	52	1
Harnaes and Seip, 1960		+•			F.	10	1
				+	M.	11	1
				+	M.	9	1
Harris, 1958				+	F.	58	1
Harrison *et alii*, 1958				+	F.	16	1
Harrison, 1959				+	M.	65	1
Helsby, 1957				+	F.	13	1
				+	F.	18	1
		+•			M.	22	1
	+				M.	48	1
				+	M.	56	1
Himel, MacDonald and Hollander, 1957				+	F.	24	1
			+		M.	18	1
Hunter and McEmoyle, 1956				+	M.	36	1
Isaac *et alii*, 1957		+•			M.	24	1
		+•			M.	41	1
Kahrs and Rømcke, 1957				+	M.	31	1
Kelly, 1958				+	F.	8	1
Kincaid-Smith *et alii*, 1958		+•			F.	12	1
				+	M.	41	1
Kincaid-Smith, 1961		+			F	51	1
	+				M.	34	1
	+				M.	45	1
				+	M	32	1
				+	M	54	1
		+•			M.	31	1
	+				M.	44	1
Latto, 1957		+•			F.	38	1
Lavender, 1957			+•		M	17	1
	+				M.	49	1
Leadbetter, 1960		+•			M.	63	2
		+•			M	58	2
Luke and Levitan, 1957	+				M.	51	3
		+•			M.	55	3
	+				M.	47	3
			+		M.	36	3
McDonald *et alii*, 1958				+	M.	59	1
Margolin *et alii*, 1957				+	M.	37	1
Mathé, 1959		+			F.	69	1
				+	M.	72	1
				+	F.	65	1
Miller and Garvan, 1956/1957				+	F.	16	1
Milloy *et alii*, 1958	+				F.	36	1
Mogg, 1957			+		M.	16	1
Mulholland, 1958				+	F.	24	1
Nesbitt, 1958				+	F.	37	1
O'Conor, 1960	+				F.	46	1
Parton and Nasbeth, 1958			+		M.	61	3
Peabody and Gates, 1958		+•			M.	56	1
Poutasse, 1959 (including Page, Dustan and Poutasse, 1959)	12	2		25	—	—	1
	2		? 1	3	—	—	5
	1		? 1		—	—	2
	3	2	? 5	9	—	—	4
Schlegel *et alii*, 1959	+				M.	18	1
	+				F.	49	1
				+	F.	34	1
				+	F.	34	4
			+		M.	25	5
			+		F.	31	1
			+		M.	8	1
	+				F.	39	1
Seige and Hauschild, 1956				+	F.	13	1
Serrato *et alii*, 1959		+•			F.	58	1
Shucksmith and Wilson, 1959			+		F.	52	2
Stamey, 1960				+	M.	41	1
Starzl and Trippel, 1959			+		M.	62	2
Stone *et alii*, 1957				+	M.	55	1
Stirling, 1957		+•			M.	53	1
Tural *et alii*, 1957		+•			F.	35	1
Ullman *et alii*, 1959				+	M.	12	1
Welch *et alii*, 1958				+	F.	7	1
			+		M.	14	1
				+	F.	11	1
				+	F.	14	1
				+	F.	7	1
			+		M.	2	1
				+	F.	11	1
				+	F.	14	1
				+	M.	13	1
				+	M.	11	1
		+•			M.	9	1
	+				F.	6	1
	+				F.	12	1
		+			F.	11	1
	+				F.	9	1

*1 = Nephrectomy. 2 = Thrombo-endarterectomy. 3 = Spleno-renal anastomosis. 4 = Other reconstructive arterial surgery. 5 = Segmental nephrectomy.

TABLE I—*Continued*

Clinical Data on 326 Cases of Hypertension Treated by Renal Surgery since 1956—Continued

Author	No Significant Reduction of Blood Pressure	Improved Blood Pressure (but Not Reduced to 140/90 mm. Hg)	Blood Pressure 140/90 mm. Hg, but Less than One Year Follow-up	Blood Pressure 140/90 mm. Hg or Lower for One Year	Sex	Age (Years)	Operation*
Wetzels and Herms, 1959	+				F.	65	1
		+			F.	45	1
Winter, 1957, and Winter *et alii*, 1959	3	6		6	—	—	1
Yates-Bell, 1959	27	18		11	—	—	1
Yendt *et alii*, 1960		+			F.	27	4
		+			M.	52	1
		+			M.	55	1
				+	M.	22	1
				+	F.	27	1
	+				M.	47	1
		+			M.	55	1
		+			M.	55	1
	+				M.	53	1
	+				M.	47	1
	+				F.	30	1
		+			M.	47	1
		+			F.	62	1
		+			F.	45	1
				+	F.	58	1
		+			F.	35	1
			+		M.	30	1
	+				M.	47	4
			+		M.	33	1
		+			F.	3	4

*1 = Nephrectomy. 2 = Thrombo-endarterectomy. 3 = Spleno-renal anastomosis. 4 = Other reconstructive arterial surgery. 5 = Segmental nephrectomy.

RESULTS OF SURGERY

The 326 cases reviewed are recorded in Table I.[1] To allow comparison with Smith's (1948, 1956) reviews, the cases are tabulated according to his criteria. No implication that a period of a year and blood pressure of 140/90 mm. Hg constitute a strict dividing line between a good and bad result is intended. Each case requires assessment of several clinical features to determine the benefit derived from operation; the effect on the systems involved is often more important than the sphygmomanometer reading. However, for purposes of tabulation, comparison of different groups and comparison with previous results, objective criteria must be used, and Smith's (1956) qualifications of established hypertension which is reduced to 140/90 mm. Hg or under for at least a year are therefore applied.

[1] Thirty-two cases recently reported by Morris *et alii* (1960) could not be included, because no details of follow-up investigation or blood pressure readings are given.

TABLE II

Pathological Lesions in Successful Cases

Pathological Lesion	Smith, 1956		Present Series	
	Number	Per centage	Number	Per-centage
Chronic pyelonephritis	62	41	23	19
Renal artery lesions ..	19	12	78	65
Hydronephrosis ..	23	15	7	6
Other lesions	45	30	12[1]	10
Total ..	149	—	120	—

[1] Other renal lesions in this series include atrophic, aplastic and hypoplastic kidneys, renal tuberculosis, papilloma and stone.

Table II shows that there has been a striking increase in the percentage of cases due to renal artery disease, which now constitute 65% of successful results compared with 12% of those recorded by Smith (1956).

Table III shows the results of operation. A blood pressure of 140/90 mm. Hg for a year was achieved in 37% of all cases; this is significantly higher than the 26% success in Smith's (1956) series. In Table I, in cases marked with an asterisk, the patients derived substantial clinical benefit from operation, although the blood pressure remained above 140/90 mm. Hg.

TABLE III

Results of Surgery in Different Ætiological Groups

Underlying Pathological Lesion	Number of Cases	Hypertension Cured on Homer Smith's Criteria		Substantial Clinical Benefit	
		Number	Per-centage	Number	Per-centage
Arterial lesions[1] ..	184	78	43	110	59
Chronic pyelonephritis	57	23	40	33	57
Other lesions[2]	85	19	22	30	35
Total ..	326	120	37	173	53

[1] All patients with renal ischæmia secondary to narrowing of the main artery or arterial branches are included under arterial lesions, except when this was due to pyelonephritis. In 10 cases trauma, surgical or external, was the apparent cause of the ischæmic lesion.

[2] This group includes hydronephrosis, atrophic, aplastic and hypoplastic kidneys, renal tuberculosis, papilloma, stone and undiagnosed lesions.

Inclusion of these additional 53 cases raised to 53% the number of cases in which a good result was obtained.

FACTORS WHICH MAY INFLUENCE THE PROGNOSIS

The Underlying Pathological Lesion

In Table III the cases are tabulated according to the underlying renal lesion, and the percentage of those in which improvement resulted from operation is shown in each group.

Most patients had renal artery lesions or pyelonephritis; hence the other groups are rather small for accurate analysis. Both renal artery disease and pyelonephritis were associated with a high percentage of successful results (43% and 40%). In the remaining 85 cases, in which there was a variety of other lesions, a reduction of the blood pressure to 140/90 mm. Hg for a year was achieved in only 22%. Operation was successful in cases of hydronephrosis, of atrophic, aplastic and hypoplastic kidneys, of renal tuberculosis, of papilloma and of stone, but individual groups are too small for accurate analysis.[1]

TABLE IV

Results of Surgery at Different Ages

Age in Years	Number of Patients	Cured on Homer Smith's Criteria		Substantial Clinical Benefit	
		Number	Per-centage	Number	Per-centage
0 to 20 ..	48	31	64	35	73
21 to 40 ..	64	23	36	40	62
41 to 60 ..	101	18	17	45	44
61 and over	21	6	28	12	56
Total	234	78	33	132	55

Age and Sex

Age appears to influence the results of surgery in renal hypertension. Table IV shows the results in 234 cases in which the individual ages were given. Of the patients aged under 20 years, 64% satisfied Homer Smith's criteria for a successful result, whereas only 17% to 36% in other age groups did so. These figures are certainly influenced by the arbitrary selection of the level 140/90 mm. Hg, which could represent residual hypertension in persons aged under 20 years. None the less, many patients aged under 20 years had malignant hypertension resistant to hypotensive drugs and showed a dramatic response to operation.

[1] Of the 27 cases of hydronephrosis, only 26% satisfied Homer Smith's criteria.

Sex did not seem to influence the response to renal surgery. An equal percentage of men and women had a successful result.

Congenital and Acquired Arterial Lesions

An analysis of the nature of the renal artery disease revealed an interesting separation of congenital and acquired lesions. Seventy-two cases in which detailed description of the lesion permitted classification are tabulated in Table V.

TABLE V

Age and Sex of 72 Patients with Renal Artery Disease

Subjects	Age (Years)	Male	Female	Total
Congenital disease: All 20 subjects aged under 41 years	0 to 10	2	1	
	11 to 20	4	2	
	21 to 30	7	2	
	31 to 41	2	—	
Total	..	15	5	20
Acquired disease: 44 of 52 subjects aged over 40 years	Under 40	4	4	
	41 to 50	13	2	
	51 to 60	14	3	
	61 to 70	11	1	
Total	..	42	10	52

All patients with congenital lesions were aged under 41 years, and the large majority with acquired lesions were aged over 40 years. Congenital lesions included cirsoid aneurysms, single or multiple arterial stenoses of a fibromuscular type and various anatomical abnormalities of arterial supply. Almost all the acquired lesions in males were athero-thrombotic, but emboli and an unusual form of arterial degeneration accounted for seven of the 10 cases in females. Among these 72 patients, 75% of the congenital lesions and 80% of the acquired lesions occurred in males. Of the total series, a similar high proportion (81%) of renal artery lesions occurred in men. Congenital lesions were associated with a higher percentage of successful results, but this is probably a reflection of the younger age of these patients.

Malignant Hypertension

The presence of papillœdema or retinitis appears to be associated with a good prognosis for cure by renal surgery.

The findings in the optic fundus were mentioned in 95 cases. Based on Homer Smith's criteria, 41% (16) of 39 patients with papillodœma and only 20% (8) of 40 patients without retinitis or papillœdema were cured. Those with retinitis but a normal optic disc formed an intermediate group, 35% of whom were cured.

Type of Surgery: Nephrectomy or Arterial Surgery

Of cases of renal artery disease, nephrectomy was carried out in 140 and arterial surgery in 44. Of those in which nephrectomy was performed, 47% fulfilled Homer Smith's criteria for a cure of hypertension, whereas in only 27% of those in which arterial surgery was done was a similar response obtained. When all cases in which substantial clinical benefit was obtained are included, a successful result was obtained in 65% after nephrectomy and in 43% after arterial surgery.

Duration of Hypertension

A group of 32 cases in which hypertension had been present for over two years did not show a significant difference in response to renal surgery. Ten (31%) of these patients fulfilled Homer Smith's criteria for a cure, including two of 10 years' duration. Fifteen (47%) derived substantial clinical benefit from operation, and in six of these the hypertension had been present for 10 to 13 years.

THE RATE OF RISE AND FALL OF THE BLOOD PRESSURE IN RENAL HYPERTENSION

In the vast majority of successful cases, the blood pressure falls immediately after operation or within a few days. However, in a few cases a slow fall may occur, taking weeks or months to reach normal levels (Harnaes and Seip, 1960; Himel and MacDonald, 1957; Howard, 1954). Douglas *et alii* (1959) record a case of malignant hypertension in a child of 10, in whom the only immediate benefit from nephrectomy was increased sensitivity to medical treatment. However, over the following two years all hypotensive drugs were withdrawn and the blood pressure remained normal. This was probably a late response to nephrectomy.

In many unsuccessful cases an immediate fall in the blood pressure is followed by a slow rise to pre-operative levels.

The rate of rise of the blood pressure is documented in several cases in which the time of a precipitating ischæmic episode is known. It may rise immediately and precipitate hypertensive heart failure within a few days (Castillo and Barrera, 1958; Gilfillan *et alii*, 1956; Götzen, 1956; McDonald *et alii*, 1958; Margolin *et alii*, 1957). In several patients who had been closely observed, the blood pressure rose to very high levels between two and three months after the ischæmic episode, having been normal or only slightly raised in the interim (Haller *et alii*, 1957; Hunter and McElmoyle, 1956; Stone *et alii*, 1957; Welch *et alii*, 1958; Yendt *et alii*, 1960).

After irradiation of the renal area, a period of six to 10 years may elapse before the patient presents with malignant hypertension (Dean and Abels, 1944; Kincaid-Smith *et alii*, 1958; Yendt *et alii*, 1960).

PATHOLOGY

On the basis of a previous autopsy study (Kincaid-Smith, 1955), it was suggested that the renal parenchymal lesion of partial infarction (ischæmic tubular atrophy) was causally related to hypertension in chronic pyelonephritis. Hypertension was thought to develop in pyelonephritis when partial infarction developed in substantial areas distal to vessels narrowed by inflammatory endarteritis. Even with identical vessel changes, no correlation with hypertension was found when fibrous scarring or "thyroid-like areas" replaced the normal renal parenchyma. It was noted that partial infarction was present in reported cases of renal artery disease in which hypertension was cured by nephrectomy. Connor *et alii* (1957) independently found an association between ischæmic tubular atrophy (partial infarction) and hypertension in cases treated by nephrectomy. A successful result could be predicted when the nephrectomy specimen showed substantial areas of partial infarction.

Since 1955, the kidneys of 15 patients on whom nephrectomy was performed in the hope of relieving hypertension have been examined. Clinical details are recorded above and elsewhere (Graber and Shackman, 1956; Kincaid-Smith *et alii*, 1958; Rosenheim, 1959; Stamey, 1960).

In two of the 15 cases the ætiology of the renal lesion was uncertain, while in six stenosis of the renal artery was present with partial infarction in the related parenchyma. In seven cases of pyelonephritis, the histological findings supported previous observations on the relationship between partial infarction and hypertension. Thus three patients who were cured of hypertension were found to have substantial areas of ischæmic tubular atrophy in the nephrectomy specimen, whereas in two who showed no improvement the renal scarring was fibrous or consisted of "thyroid-like" tissue without ischæmic areas. In the remaining two, in whom some improvement occurred, mixed scarring was present. In 12 of the other 319 cases reviewed, removal of a pyelonephritic kidney did not alter the blood pressure. Histological descriptions are available in five of these, and the scarring was of the "non-ischæmic" type without areas of partial infarction.

Among cases of stenosis of the renal artery or pyelonephritis, Case IV is the only one I have

seen in which the blood pressure did not fall after removal of a kidney containing large ischæmic areas. A month later at autopsy, examination of the other kidney revealed equally extensive areas of partial infarction distal to narrowed interlobular arteries, the result of long-standing malignant hypertension. This ischæmic tissue presumably maintained the blood pressure at a high level after nephrectomy.

It may be important to recognize ischæmic changes in the kidney at operation. Removal of only the ischæmic area by partial nephrectomy may cure hypertension (Poutasse, 1959). On

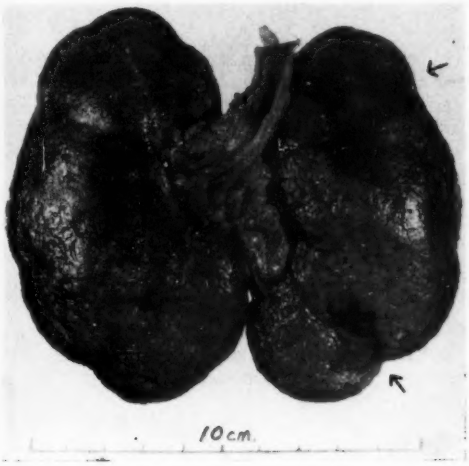

FIGURE I

A kidney in which two branches of the renal artery were occluded. Arrows indicate resultant smooth dark ischæmic areas sharply demarcated from the normal renal cortex

the other hand, if ischæmic changes are present in both kidneys, nephrectomy is contraindicated. Ischæmic renal parenchyma usually appears smooth, dark and depressed below the surface compared with normal cortex. A sharp line of demarcation is usually present along the line of distribution of a branch of the renal artery (Figure I).

Although renal arteries appear on injection studies to be end arteries (Graves, 1956; More and Duff, 1951), complete occlusion of the main renal artery may be found in association with surviving renal parenchyma (Adams and Newman, 1958; Birch-Jensen and Røjel, 1958; Stone *et alii*, 1957; McDonald *et alii*, 1958). The ischæmic lesion in such cases is usually patchy, presumably owing to revascularization of some areas via capsular or peripelvic vessels, as occurs in animals (Sheehan and Davis, 1959). Recovery of function in animals with a ligated renal artery may occur through revascularization via either a blindly implanted splenic artery (Davis and Morse, 1957) or an attached skin pedicle (Stone *et alii*, 1956). This can be explained only on the basis of collateral supply, and in my experience large capsular and pelviureteric anastomotic channels are a prominent feature in cases of main renal artery occlusion.

Hypertensive Vessel Changes

Interlobular artery narrowing secondary to malignant hypertension producing partial infarction in related areas of cortex is associated with coarse surface nodularity, and if present in the opposite kidney is a contraindication to nephrectomy. Only some patients with malignant hypertension will show this change, and since it is usually associated with a high blood urea level (Kincaid-Smith *et alii*, 1958) surgery is not contemplated. Apart from this change, none of the other histological vessel changes which result from hypertension seems to prevent a response to nephrectomy. Thus the fibrinoid arteriolar lesions of malignant hypertension and all grades of benign hypertensive vessel changes may be seen in cases in which a satisfactory response is obtained. Hypertensive vessel changes are usually less severe in ischæmic areas of kidney, though hypertensive changes including fibrinoid necrosis may be found distal to definite renal artery narrowing; thus arterial stenosis does not invariably "protect" the vessels from the effects of the hypertension.

The juxtaglomerular apparatus is frequently hypertrophied in renal parenchyma distal to an arterial stenosis. Recent work indicates that this is probably the site of manufacture of renin (Tobian, 1960).

DISCUSSION

The results of renal surgery for the treatment of hypertension have improved steadily over the past two decades. Smith *et alii* (1943) found that in only 9% of the 76 cases then on record was a successful result obtained. Smith's (1948, 1956) two further reviews showed a rise to 19% of 242 operations in 1948 and 26% of 575 operations in 1956. The present series recorded since that time shows a further improvement to 37% success even when Smith's criteria are applied. It is difficult to assess the actual benefit derived from renal surgery. A follow-up period of a year is a reasonable requirement, because an immediate fall in the blood pressure is often followed by a slow rise to pre-operative levels over several months. It is far more difficult to select a level below which the patient

is considered "cured". Any such level is arbitrary, and even if the patient does not satisfy this requirement considerable clinical benefit may result from operation. The level 140/90 mm. Hg suggested by Smith (1948) has been adopted to permit comparison with previous figures, and because an objective criterion must be used in order to compare results in different groups of cases. In addition, an attempt was made to assess actual clinical benefit from individual case records, and inclusion of a further 53 cases brings to 173 (53%) the total number who derived substantial benefit from the operation. Thus in about half the cases reported worthwhile benefit has been derived from operation; but this may be an optimistic estimate (of the actual chances of success), because of the tendency to report good results rather than failures.

It is likely that the steady improvement in the results of surgery for hypertension is due largely to better selection of cases. Improvement in surgical technique is not likely to have influenced results; nephrectomy has altered little in recent years, and arterial surgery, which is developing rapidly, is as yet less likely to achieve a successful result than nephrectomy. Better selection of patients involves exclusion of those with bilateral disease unless bilateral arterial lesions can be corrected, and in addition there has been a strong tendency in the past four years to restrict surgery to patients most likely to improve—namely, those with renal artery lesions and pyelonephritis. Previous experience indicated that the chances of relieving hypertension were higher in such cases than in a large variety of other conditions, such as hydronephrosis, calculi, pyonephrosis, hypernephroma, cysts and tuberculosis (Braasch, 1942; Langley and Platt, 1947; Thompson and Smithwich, 1952; Thompson, 1957; Gellman, 1958; Harris, 1958; Smith, 1956). In the present review, the percentage of cases of renal artery disease and pyelonephritis with a successful result from surgery was almost double that found in other renal lesions.

The nature of the underlying pathological lesion should always be taken into account when surgery is being contemplated in renal hypertension. Even in renal arterial disease and pyelonephritis, a good clinical result can be anticipated in only about half the cases. This must be weighed against operative risks and against constant improvements in medical treatment. When nephrectomy is not successful it may hasten the progress of the disease and precipitate uræmia, and although arterial surgery may avoid loss of renal parenchyma, it probably carries a higher operative risk, particularly in older patients with degenerative vascular disease in other organs (Page *et alii*, 1959). Renal arterial surgery is still in its infancy, and some very good results (De Camp and Birchall, 1958; Poutasse, 1959; Brown *et alii*, 1960; Morris *et alii*, 1960) suggest that in the future it is likely to be associated with a higher rate of success than is shown by present figures. Nonetheless, those with experience in this field treat some patients with proven arterial lesions with hypotensive drugs in preference to surgery.

In accord with other observations (Schaffer and Markowitz, 1954; Yates-Bell, 1959), patients aged under 20 years appear to be more likely to benefit from renal surgery than those in other age groups. This is in part a reflection of selecting the level 140/90 mm. Hg as a dividing line, since this may represent residual hypertension in young persons. However, there is no doubt that operation was life-saving in many young patients with malignant hypertension resistant to hypotensive drugs. The alternative, even when medical treatment is effective, is a lifetime of careful supervision with some unpleasant side effects of medication, and when surgery is feasible it is the treatment of choice at this age. In patients aged over 50 years, in whom renal hypertension is likely to be due to athero-thrombotic arterial lesions, associated degenerative vascular disease in other organs is responsible for a high post-operative mortality rate (8% early and 9% late post-operative deaths—Page *et alii*, 1959). Thus hypotensive drugs, if effective, are probably the treatment of choice for older patients.

In patients aged under 40 years, renal artery disease is likely to be due to a congenital lesion; but over this age, particularly in men, it is almost always athero-thrombotic, being similar in appearance to occlusive coronary lesions which are common in men at this age.

Hypertension of long duration does not necessarily diminish the likelihood of response to operation. Six of seven patients in whom hypertension had been present for 10 to 13 years had a good response. It is often said that long-standing hypertension causes irreversible vascular lesions in the opposite kidney, which maintain the blood pressure at a high level; but there are many cases on record in which long-standing hypertension has been relieved by nephrectomy (Sensenbach, 1944; Langley and Platt, 1947; Kilman *et alii*, 1949; Schaffer and Markowitz, 1954; Poutasse, 1956; Harris, 1958; Welch *et alii*, 1958; Ullmann *et alii*, 1959). In five cases quoted by Schaffer and Markowitz (1954), hypertension which had been present for 20 to 23 years was cured by nephrectomy.

The vascular changes in the nephrectomy specimens examined support previous observations (Pickering *et alii*, 1952; Thompson and Smithwick, 1952), that neither severe benign nor malignant (fibrinoid necrosis) hypertensive arteriolar lesions prevent a fall in the blood pressure to normal levels after nephrectomy. When interlobular artery narrowing occurs as a result of malignant hypertension, secondary areas of ischæmic atrophy appear in the related renal cortex (Kincaid-Smith *et alii*, 1958). Since ischæmic atrophy is associated with the development of hypertension when larger renal arteries are stenosed, it is reasonable to assume that it will prevent a response to nephrectomy if present in the opposite kidney as a result of interlobular artery narrowing. Most patients with malignant hypertension associated with interlobular artery narrowing have a raised blood urea level, so nephrectomy is seldom contemplated; but the blood urea level may be borderline, and nephrectomy may precipitate uræmia, as in Case IV.

In spite of this observation, the likelihood of a good response to nephrectomy seems to be higher in patients with papillœdema and retinitis than in those with less marked hypertensive retinal changes. This may reflect the fact that true renal hypertension is often of the accelerated type, and that the renal disease present in some of the less severe cases was incidental.

Examination of nephrectomy specimens and descriptions of other recorded cases support the hypothesis that it is the ischæmic partially infarcted- renal parenchyma in chronic pyelonephritis which is related to the development of hypertension in this condition.

The question of whether hypokalæmia may result from ischæmic renal lesions was raised by Dollery *et alii* (1959), who reported Case II in detail, together with a similar case. Recently Laragh and his colleagues (1960) have shown that aldosterone secretion is raised in malignant hypertension, and thus it is possible that it was the cure of the malignant hypertension which stopped the excessive potassium excretion after nephrectomy in Dollery's two cases. However, I have observed six additional patients presenting with hypokalæmia, all of whom had the common feature of extensive partial infarction in the kidney. Four of these had malignant hypertension, but two had polyarteritis nodosa without malignant hypertension, and none showed any suprarenal lesion at autopsy. It seems, therefore, that either ischæmic renal parenchyma constitutes one type of "potassium-losing kidney", or that in these cases there was a common feature stimulating high aldosterone secretion. The hypertrophied juxtaglomerular apparatus found in ischæmic kidneys may provide the link, as this is the supposed site of renin formation, which in turn may stimulate aldosterone secretion (Genest *et alii*, 1960).

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RENAL BIOPSY

WITH PARTICULAR REFERENCE TO THE STUDY OF DIABETES MELLITUS, SYSTEMIC LUPUS ERYTHEMATOSUS AND SUBACUTE GLOMERULONEPHRITIS[1]

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SUMMARY

In this study diagnostically adequate tissue was obtained by aspiration biopsy of the kidney in 200 (78%) of the 257 patients investigated. Diabetic nephropathy most often complicated long-standing diabetes; but diabetic renal lesions did not depend on the duration and severity of the diabetes or on the degree of diabetic control with insulin. Twenty-five patients with lupus nephritis were treated with cortisone; "mild" and "moderate" glomerular lesions showed histological arrest; but improvement was exceptional. Histological study of 38 cases of subacute glomerulo-nephritis supported the validity of Allen's (1955) classification. In a limited analysis, the long-term prognosis of patients treated with cortisone was not significantly better than that of untreated cases.

A fluorescent antibody reagent was applied to a portion of the renal biopsy specimen in 71 cases, in order to determine the possible part played by antibodies in the production of nephritis. Positive reactions, suggesting localization of human globulin in the glomerulus, were obtained chiefly in lupus nephritis and in diffuse membranous glomerulo-nephritis.

Renal biopsy proved of most value in early diffuse renal disease wherein diagnosis, prognosis and probable response to treatment are best assessed on morphological grounds; it was of less value in advanced renal disease with sclerosis.

THIS present report gives our impressions of the value and limitations of renal biopsy, and the information gained from the procedure in 200 cases.

METHODS

The patients studied were over the age of 14 years and had been admitted to the Royal Melbourne Hospital. Biopsy was performed by a posterior approach, and our only absolute contraindications were bleeding diatheses and single functional kidneys; biopsies were performed in cases of anuria, uræmia and hypertension, although these were regarded as relative contraindications.

The biopsy fragment was immediately fixed in 10% formol-saline solution and embedded in paraffin; serial sections 5μ in thickness were cut to reduce the sampling error and stained with hæmatoxylin and eosin. Van Gieson and periodic acid-Schiff (PAS) stains were used to assess fibrosis and glomerular basement membrane changes. For the fluorescent antibody studies, frozen sections were cut from a gelatine-embedded cortical fragment, transferred to slides coated with egg albumin dried *in vacuo*, and stained with rabbit antihuman gamma-globulin antibody coupled with fluorescein.

RESULTS

Success Rate and Complications

Biopsy fragments were obtained from 200 (78%) of the 257 patients studied, and in (229 (68%) of 334 separate biopsy attempts (Table I). These figures represented the experience of several individuals; in single practised hands better figures are being obtained. The average number of glomeruli in the biopsy specimen was 17 in an analysed series of 52 biopsies. Biopsy fragments containing five or more glomeruli were considered satisfactory.

Hæmorrhage occurred in 14 patients, six of whom had hypertension.[1] Perirenal bleeding occurred in six cases, five patients requiring blood transfusion, and gross hæmaturia in eight cases, two patients requiring transfusion. In two patients perirenal bleeding was more severe and protracted. One was gravely ill

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[1] Blood pressures exceeding 180 systolic or 100 diastolic mm. Hg.

TABLE I

Patients Studied, Success Rate, Complications and Reproducibility of Renal Biopsy

Diagnoses	Success Rate of Renal Biopsy				Post-Biopsy Hæmorrhage		Comparison of Biopsy with Subsequent Sample			
	For Individual Patients		For Biopsy Attempts							
	Successful	Total	Successful	Total	Perirenal	Gross Hæmaturia	Total Number	Similar	Pro-gression	Dis-similar
Diabetes mellitus[1]	68	80	72	98	0	3	7	2	5	0
Systemic lupus erythematosus	22	28	33	48	1	1	14	10	4	0
Subacute nephritis	38	45	50	65	1	2	15	7	8	0
Nephrotic syndrome (minor causes)[2]	8	9	8	9	0	0	1	1	0	0
Acute nephritis	6	9	6	9	0	0	0	0	0	0
Chronic nephritis	12	20	12	32	1	2	3	0	0	3
Pyelonephritis	9	10	9	12	0	0	2	1	0	1
Essential hypertension ..	8	10	9	11	1	0	3	3	0	0
Oliguria, anuria	7	7	7	8	1	0	3	3	0	0
Miscellaneous renal disease ..	8	8	9	11	1	0	4	4	0	0
Miscellaneous non-renal disease	14	31	14	31	0	0	0	0	0	0
Total	200	257	229	334	6	8	52	31	17	4

[1] Including four cases of hæmochromatosis.
[2] Including nephropathy of pregnancy, amyloidosis and renal vein thrombosis.

with proliferative glomerulo-nephritis, hypertension and uræmia, and died of renal failure seven days after the biopsy; the other had scleroderma, hypertension and coronary atherosclerosis, and died 18 hours after biopsy. In both, autopsy revealed a large perirenal hæmatoma, estimated at 1200 to 1500 ml., but no tearing of the kidney (Figure I). In two cases a perirenal abscess occurred subsequent to biopsy.

FIGURE I

Kidney at necropsy from patient with scleroderma, in whom biopsy was complicated by a perirenal hæmatoma; there is a small puncture wound and no tearing of the kidney

Diagnostic Value of Renal Biopsy

Fifty-two biopsy specimens were compared with renal tissue subsequently obtained by biopsy in 21 cases and at autopsy in 31 cases (Table I). The histological changes were similar in the two specimens in 31 cases, and similar but with evidence of progression of the disease in 17 cases. The histological changes were dissimilar in only four cases, in all of which "chronic nephritis" was present, suggesting that "sampling" error was not a significant problem in the diffuse renal diseases included in this study.

Laboratory tests were compared with biopsy specimens from 70 patients with diabetes or systemic lupus erythematosus (S.L.E.); in 35 the glomeruli were histologically normal and in 35 the glomeruli were abnormal. The patients in the former group all gave normal results to urea tests[1]; but three patients had unexplained proteinuria or an abnormal sediment; mild vascular sclerosis was found in two of these biopsy specimens. Of the 35 patients with abnormal biopsy findings, only 24 had proteinuria or an abnormal sediment and 19 gave abnormal results to urea tests; in five cases in which well-defined glomerular lesions were present, all laboratory tests gave repeatedly normal results. These findings indicated the sensitivity of renal biopsy as an index of early glomerular disease.

The Nephrotic Syndrome

Fifty-three of our 200 patients suffered from the nephrotic syndrome. The cause was diabetic nephropathy in seven, lupus nephritis in seven, subacute glomerulo-nephritis in 32, toxæmia of pregnancy in four, amyloidosis in two and renal vein thrombosis in one. It is not proposed to discuss histological findings in the nephrotic

[1] Urea tests here refer to the blood urea level, for which the normal range is 15 to 40 mg. per 100 ml. of blood, and the Fowweather urea clearance test, wherein values of less than 70% of "average normal function" are considered abnormal (Maxwell, 1947).

syndrome in general; the major causes were usually readily distinguished on clinical grounds prior to biopsy assessment of the lesion.

Diabetes Mellitus

The first 54 biopsy specimens were obtained from 50 unselected consecutive diabetic patients; the biopsy findings were normal in 20, and showed vascular disease in nine and diabetic renal decease in 21 (Table II). Subsequently, 18 biopsies were performed on a further 17 patients because of proteinuria and hypertension; diabetic nephropathy was found in 12 and vascular changes in five only.

In early diabetic nephropathy, two types of glomerular lesion — "diffuse" and "exudative"—were recognized (Taft *et alii*, 1954). Diffuse lesions began as localized acidophilic thickenings of the basement membrane of the capillary walls; these subsequently became diffuse and caused capillary occlusion and glomerular sclerosis. Exudative lesions appeared as deposits situated adjacent to the capillary tufts or in the capsular basement membrane, and gave the staining reactions of glycoprotein and lipid; the staining reactions of the exudative lesions were similar to those of the tubular casts and the pronounced hyaline arteriosclerosis which accompanied advanced diabetic nephropathy. When the exudative material became walled off by argyrophilic material and collagen formation, the staining reactions tended to resemble those of the diffuse lesions. The progressive development of diffuse and exudative lesions ultimately combined to produce the "nodular" lesion characteristic of diabetic renal disease (Figure II). Nodular lesions were seen only in biopsy specimens from patients with "senile" and long-standing "juvenile" diabetes.

In diabetes, abnormal biopsy appearances were more frequently seen in females, in older patients with long-standing disease, and in patients with diastolic hypertension, cardiovascular disease and retinopathy (Table II). Pathological lesions occasionally preceded clinical manifestations of the Kimmelstiel-Wilson syndrome by long intervals, and in three cases specific glomerular changes were present in the absence of any clinical or laboratory indication of renal disease; in two cases lesions were found within three months of the clinical onset of

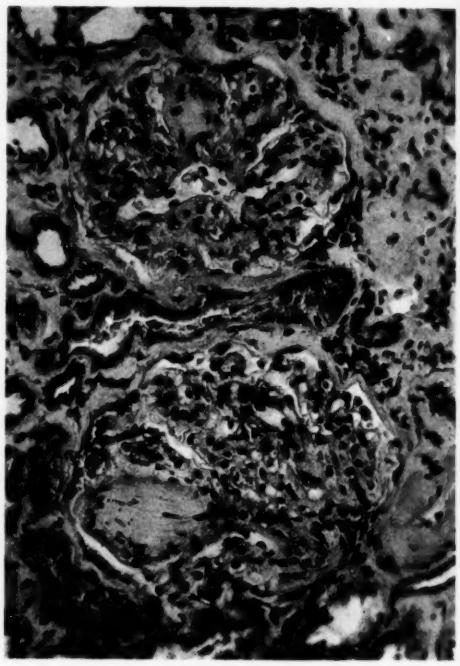

FIGURE II

Classical advanced diabetic nephropathy in a female, aged 50 years, with diabetes mellitus for 20 years, retinopathy, neuropathy, proteinuria and renal insufficiency. The glomeruli show the pathognomonic "nodular" lesions and also diffuse lesions of the capillary walls. (×200)

TABLE II

Relationship of Renal Biopsy Appearances to Clinical Features in Diabetes Mellitus—Case Distribution[1]

Histological Diagnosis	Number of Patients	Mean Age (Years)	Mean Duration of Diabetes (Years)	Juvenile or "Brittle" Cases	Poor Diabetic Control[2]	Diastolic Hypertension	Cardio-vascular Disease[3]	Retinopathy
Normal renal tissue	20	29	5	13	7	0	1	2
Renal vascular disease[4]	9	57	13	2	2	3	8	5
Diabetic renal disease	21	44	11	12	2	7	11	8

[1] Based upon biopsies from 50 consecutive unselected diabetic patients (Taft, Taft and Joske, 1960).
[2] Frequent glycosuria and episodes of ketosis.
[3] Myocardial ischæmia, peripheral arteriosclerosis, cerebro-vascular disease.
[4] Atheroma and arteriosclerosis.

diabetes. The occurrence of diabetic nephropathy did not correlate with the degree of control of hyperglycæmia with insulin (Table II), and subsequent biopsies on four patients with established renal disease showed progression of the glomerular lesions in three despite good control of the diabetes.

Systemic Lupus Erythematosus

Thirty-three needle biopsies were performed on 22 patients[1] with systemic lupus erythematosus, including 11 previously described by Joske and Stubbe (1957) (Table III). There were 23 females and two males, aged between 18 and 59 years. The series included three patients with lupoid hepatitis. All patients received continuous treatment with cortisone[2] in a dosage sufficient to control most symptoms, this varying from 50 to 175 mg. (or its equivalent) daily. Our criteria for including patients in this group were the unequivocal finding of L.E. cells in association with at least one visceral lesion in keeping with S.L.E.

Histological Lesions of Lupus Nephritis.—In "mild" lesions (Figure III), the process was initially focal within both the kidney and the individual glomeruli. The early focal lesion consisted of thickening of the basement membrane, which stained intensely with PAS, and mild endothelial hyperplasia.

"Moderate" lesions (Figure IV) showed more diffuse involvement of the basement membrane of the tufts ("wire-loops"), which were strikingly eosinophilic and refractile, and also pronounced endothelial hyperplasia, capillary narrowing and fine capsular adhesions.

"Advanced" lesions (Figure V) showed capillary occlusion, nodularity and confluence of the tufts, dense adhesions, epithelial swelling

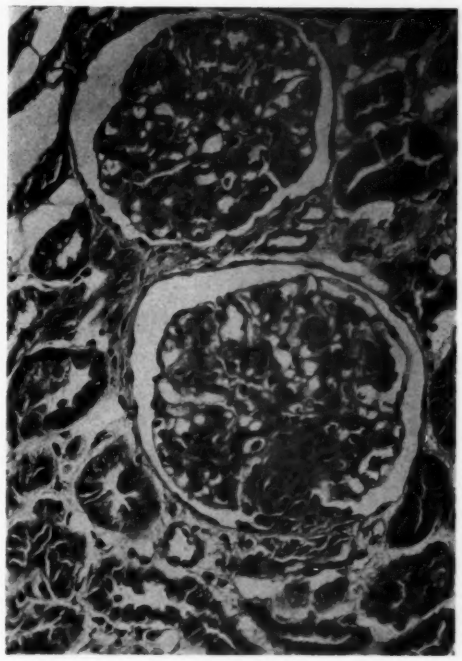

FIGURE III

A female, aged 23 years, with systemic lupus erythematosus, but normal urinary findings. Glomeruli show slight patchy thickening of capillary basement membranes and foci of endothelial hyperplasia: mild lupus nephritis. (High power. ×200)

[1] Three additional patients were studied on whom needle biopsy was not performed.

[2] "Corticosteroids" in this paper refers to cortisone and the newer derivatives.

TABLE III

Histological and Clinical Features of 25 Cases of Systemic Lupus Erythematosus and Lupus Nephritis—Case Distribution

Histological Diagnosis at Initial Biopsy	Number of Cases	Mean Duration of Symptoms Prior to Initial Biopsy (Months)	Mean Duration of Illness (Years)[2]	Clinical Features at Initial Biopsy			Daily Maintenance Dose of Cortisone		Course of Renal Lesion[1]				Death[4]	
				Œdema	Hypertension	Azotæmia	50–100 mg.	>100 mg.	No Lesion or Arrest	Progression			Renal Failure	Other Causes
										Slow	Moderate	Rapid		
Normal kidney	6	36	7·5	0	0	0	4	2	4**	1*	1*	0	0	0
Interstitial nephritis	3[3]	84	10	0	0	0	1	2	3**	0	0	0	0	2[ab]
Glomerulitis:														
Mild	5	28	4	0	0	0	3	2	3	1*	0	1*	1	1[c]
Moderate ..	6	24	5	2	3	2	3	3	4*	1*	1	0	0	3[add]
Advanced ..	5[4]	22	3·5	4	5	5	2	3	1	0	1*	3***	4	0

[1] Asterisks indicate cases in which serial biopsies and/or necropsy were obtained.
[2] Until death or to June, 1960.
[3] Includes one surgical biopsy.
[4] Includes two cases studied at necropsy in whom biopsy was not performed.
[5] *a*, cardiac failure; *b*, traffic accident; *c*, hepatic failure; *d*, cause unknown, possibly adrenal insufficiency.

and proliferation, and even crescent formation; exudate and erythrocytes often occupied the remaining capsular space. Ultimately periglomerular fibrosis accompanied these ischæmic lesions, the end result being a sclerosed glomerulus

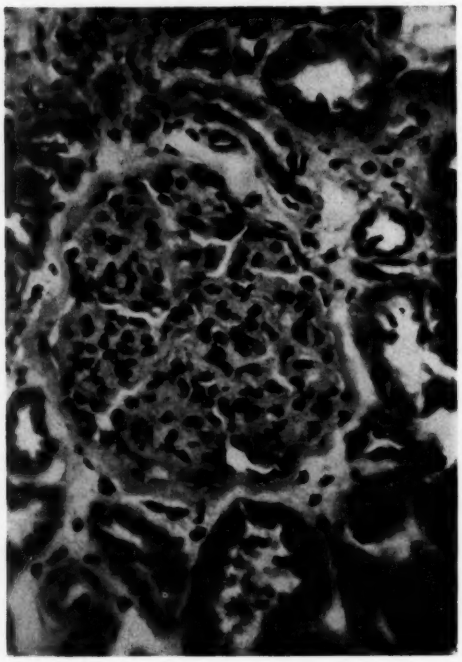

FIGURE IV

A female, aged 26 years, with systemic lupus erythematosus, proteinuria and hypertension. There is diffuse basement membrane thickening, endothelial hyperplasia, moderate capillary occlusion: moderate lupus nephritis. (High power. ×275)

surrounded by collagen. Specific glomerular lesions, such as hæmatoxylin bodies and granular eosinophilic necrosis of the tufts, were seen only in three severely affected kidneys.

The tubular and interstitial changes were not specific. In the advanced disease, hypertensive arteriosclerotic changes were prominent in the arterioles and interlobular arteries. Lymphocytes, plasma cells and sometimes polymorphs infiltrated the interstitial tissue and occasionally formed dense periglomerular aggregates. In three cases glomerular lesions were slight or absent, but there were the pronounced interstitial cellularity, fibrosis and tubular casts suggestive of pyelonephritis; this severe interstitual lesion possibly represented a variant of lupus nephritis.

Clinico-Pathological Correlations (*Table III*).—In six patients the initial biopsy findings were normal. Four escaped renal damage, but in the other two serial biopsies showed that lupus nephritis had supervened despite daily maintenance dosages of prednisolone of 20 to 25 mg.; the nephritis became arrested in one, but progressed in the other patient.

In five patients, the glomerular lesions were mild. Three remained in clinical remission with arrested nephritis; two patients died, one from liver failure (Taft *et alii*, 1958), and one from progressive nephritis and renal failure after cortisone was withdrawn.

In six patients the nephritis was of moderate severity. Two had a nephrotic syndrome, three

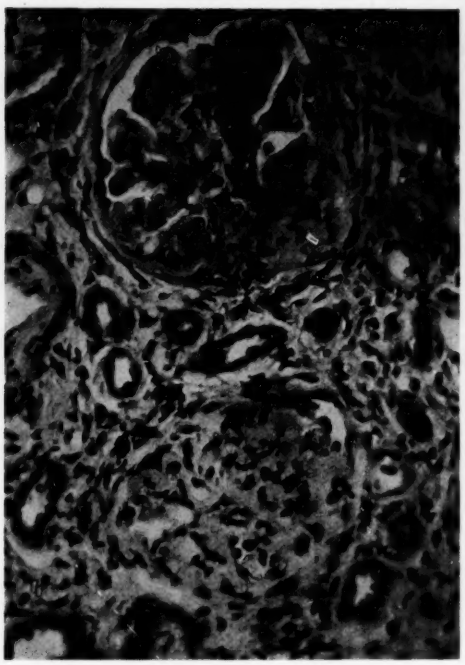

FIGURE V

A female, aged 34 years, with systemic lupus erythematosus, nephrotic syndrome, hypertension and renal insufficiency. The biopsy shows two glomeruli with pronounced basement membrane thickening, hypercellularity of the tufts, glomerular adhesions, capillary occlusion, interstitial fibrosis and tubular atrophy: advanced lupus nephritis. (High power, ×200)

were hypertensive and two had impaired renal function. Cortisone induced a clinical remission in five cases. The nephritis has remained arrested in three cases, and slow progression with supervening pyelonephritis occurred in

one. Two patients, including one who failed to improve with cortisone, died suddenly at home; adrenal insufficiency was possibly implicated in both.

Three patients showed advanced glomerular lesions and clinical and laboratory evidence of severe renal disease; two other patients had severe nephritis clinically, and biopsy was deferred. Of this group, four have died of renal failure; cortisone was not of benefit and aggravated preexisting hypertension and fluid retention.

Glomerulitis did not occur in the three patients with interstitial nephritis, but two died of non-renal causes.

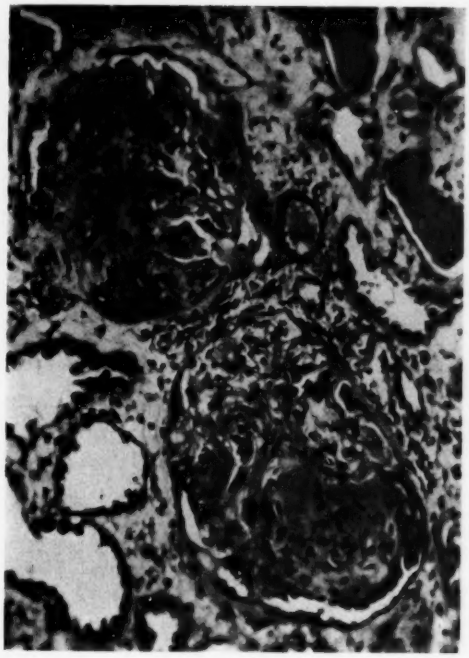

FIGURE VI

A male, aged 26 years, with acute post-streptococcal nephritis 14 years previously, nephrotic syndrome, hypertension and pulmonary hæmorrhages. The glomeruli show epithelial hyperplasia and fibrosis, inflammatory infiltration, adhesions and capillary occlusion: progressive acute nephritis. (High power. ×200)

Subacute Glomerulo-nephritis

The term "subacute glomerulo-nephritis" denoted the phase of glomerulo-nephritis characterized clinically by massive proteinuria; 32 of the present 38 patients had a nephrotic syndrome, whereas six had only proteinuria. Cases in which hypertension and nitrogen retention were present were referred to as being "mixed" or "complicated", as distinct from "pure" or "uncomplicated" (Allen, 1955; Johnson and Reader, 1959). The glomerular changes were classified histologically into sub-

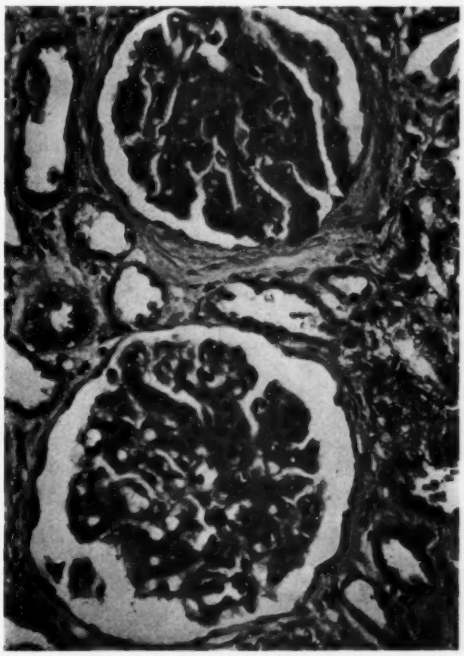

FIGURE VII

A male, aged 46 years, with a nephrotic syndrome for two and a half years. The glomeruli show diffuse basement membrane thickening: diffuse membranous glomerulo-nephritis. (High power. ×200)

groups according to Allen (1955) and Kark *et alii* (1958*b*); the tubular changes were non-specific.

Progressive Acute (Proliferative) Glomerulo-nephritis.—This condition (four cases) corresponded to Type I nephritis of Ellis (1942), and showed mainly epithelial proliferation in the biopsy specimen. The glomerular changes were pronounced and generalized, and included infiltration with polymorphs and mononuclear cells, endothelial hyperplasia, epithelial crescents, basement membrane disruption, capsular adhesions and capillary occlusion (Figure VI).

Membranous Glomerulo-nephritis. — Membranous glomerulo-nephritis (31 cases) corresponded to Type II nephritis of Ellis (1942) and was histologically sub-grouped as follows:

(1) Diffuse membranous glomerulo-nephritis (11 cases), wherein there was a characteristically

uniform eosinophilic thickening of the basement membrane, advanced degrees of which caused capillary occlusion (Figure VII). Occasionally, focal thickenings and a slight increase in endothelial cells made differentiation from lupus nephritis difficult.

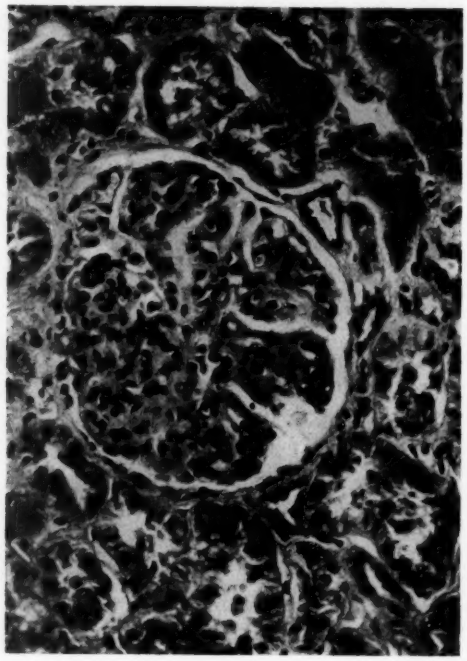

FIGURE VIII

A female, aged 15 years, with nephrotic syndrome for five months. The glomeruli show basement membrane thickening associated with hypercellularity mostly due to endothelial hyperplasia: membranous proliferative glomerulo-nephritis. (High power. ×250)

(2) Membranous - proliferative glomerulo-nephritis (17 cases), in which basement membrane thickening and, in addition, focal endothelial proliferation and fine capsular adhesions were present (Figure VIII). This group included cases of focal nephritis as described by Heptinstall and Joekes (1959). Advancing changes included epithelial swelling and proliferation, splintering of the basement membrane, occlusion of capillaries and intraglomerular and peri-glomerular fibrosis.

(3) Lobular membranous glomerulo-nephritis (three cases), which represented a severe and generalized glomerular lesion; pronounced nodular thickening of the basement membrane and considerable endothelial hyperplasia caused fusion and pseudolobulation of the tufts, capillary occlusion and glomerular ischæmia (Figure IX).

Normal glomeruli were seen in three cases despite heavy proteinuria. Changes involving the epithelial foot processes in the glomeruli have been demonstrated in such cases by electron microscopy (Farquhar *et alii*, 1957).

Clinico-Pathological Correlations (*Table IV*).—The ages of the patients ranged from 16 to 75 years, and the mean age was 28 years in proliferative glomerulitis and ranged from 35 to 40 years for the sub-groups of membranous glomerulo-nephritis. The high incidence in middle-aged males was striking. The mean duration of symptoms before biopsy was undertaken ranged from seven to 22 months.

All four patients with proliferative glomerulo-nephritis had suffered from antecedent acute

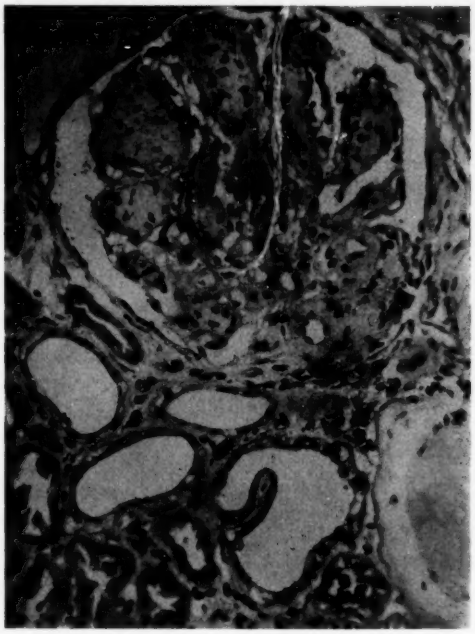

FIGURE IX

A male, aged 66 years, with severe nephrotic syndrome for two years. The biopsy shows the characteristic lobulation of the tufts: lobular membranous glomerulo-nephritis. (High power. ×300)

nephritis or chronic streptococcal infection. Most patients with membranous glomerulo-nephritis gave no history of preceding disease, although six had septic infections or febrile illnesses within weeks of the onset of œdema.

TABLE IV

Histological and Clinical Features of 38 Cases of Subacute Nephritis—Case Distribution

Histological Diagnosis at Initial Biopsy	Number of Patients	Sex M.	Sex F.	Mean Age (Years)	Mean Duration of Symptoms (Months)[1]	Clinical Syndrome at Initial Biopsy: Pure	Mixed: Ht	Mixed: Az	Mixed: Ht[2] Az	Antecedent Illness or Suspected Ætiology[3]	Assessment of Course of Disease: Clinical[4] R	Clinical[4] U	Clinical[4] D	Not Followed	Histological[5]: Arrest	Histological[5]: Progression
Proliferative glomerulonephritis	4	4	0	28	15	0	3	4	4	4^{aaaa}	0	1	3	0	0	3
Membranous glomerulonephritis:																
(i) Diffuse	11	8	3	35	18·5	5	3	6	6	4^{abcc}	3	3	3	2	1	2
(ii) Membranous - proliferative	17	11	6	39	7	9	3	7	7	3^{dee}	6	2	6	3	2	5
(iii) Lobular	3	3	0	40	22	2	0	1	1	2^{ce}	0	0	3	0	0	3
Normal glomeruli	3	2	1	40	10	3	0	0	0	2^{ef}	2	0	1	0	1	1
Total	38	28	10	37	12	19	9	18	18	15	11	6	16	5	4	14

[1] Until initial biopsy.
[2] Ht, hypertension; Az, azotæmia.
[3] *a*, streptococcal throat infection; *b*, streptococcal wound infection; *c*, lead exposure; *d*, dental sepsis; *e*, "febrile illness"; *f*, cholangitis.
[4] R, remission; U, unchanged; D, deterioration.
[5] By serial biopsy and/or necropsy.

Three patients with membranous glomerulonephritis had industrial exposure to lead; in one, who presented with frank lead poisoning and minimal proteinuria, there was mild basement membrane thickening in seven of 17 glomeruli.

A "pure" or uncomplicated nephrotic syndrome existed in only 19 of our 38 patients with subacute glomerulo-nephritis at the time of the initial biopsy. This high incidence of "mixed" nephrotic syndromes, which increased during the period of observation, can be attributed to the adult sample and to the frequently long interval from the onset of illness to the initial biopsy.

The course was assessed as "remission", "unchanged" wherein œdema was continuous or recurring, and "deterioration". Histological reassessment was possible by biopsy in 11 and by necropsy in seven of the 38 cases. Remission was usually associated with histological arrest, but in only two cases did histological improvement occur. However, serial biopsies showed histological deterioration and supervening glomerulo-sclerosis in two patients in apparent clinical remission. Clinical deterioration always corresponded with histological deterioration.

The groups of cases of diffuse membranous and membranous-proliferative nephritis behaved similarly in relation to course, suggesting that endothelial proliferation, in addition to basement membrane thickening, did not affect prognosis; of 23 adequately followed patients in these two groups, 10 had a sustained clinical remission, the condition of seven remained unchanged, and six deteriorated. The three patients with lobular lesions all deteriorated, illustrating the poor prognosis of this lesion.

The patients with proliferative glomerulonephritis and with normal glomeruli were too few in number for analysis. It should be noted that one patient with normal glomeruli on biopsy remained œdematous despite treatment with cortisone, and died of pulmonary œdema; necropsy showed typical changes of membranous glomerulo-nephritis.

Cortisone treatment was assessed solely on a long-term basis over a minimal period of two years; treatment was usually continuous, but occasionally intermittent. The patients who were not treated with cortisone, although not a randomly allocated control group, may be used for purposes of comparison.

The 29 treated and untreated patients were subdivided according to the histological findings and according to whether the disease was complicated by hypertension or aztoæmia (Table V). In neither of these subdivisions was long-term cortisone treatment shown to be of clear advantage. In all, remission with sustained histological arrest occurred in only eight of the 20 treated patients as against three of the nine untreated patients, the untreated patients thus behaving similarly to the treated patients.

Fluorescent Antibody Studies

The results obtained in 71 cases of diffuse renal disease are presented in Table VI. Twelve patients with systemic lupus erythematosus were studied; in eight of nine patients whose biopsy showed renal disease fluorescence of

glomerular capillary loops occurred, and in the other the lesions were mild. In three cases the biopsy findings were normal and fluorescence did not occur. In one patient with lupoid hepatitis, glomerulo-nephritis and ulcerative colitis (Taft *et alii*, 1958) the glomeruli fluoresced, providing further evidence that the renal lesion resembled that of lupus nephritis.

TABLE V

Treatment of Subacute Glomerulonephritis with Cortisone and Derivatives—Long-term Results

Histological Subtypes; Presence of Complications[1]	Treatment with Cortisone[2]	Number of Cases.	Course of Disease[3]: Remission, Histological Arrest	Recurring Œdema	Deterioration, Histological Progression
Membranous glomerulo-nephritis:					
Diffuse ..	+	4	1	1	2
	−	5	2	2	1
Membranous-proliferative	+	10	5	0	5
	−	4	1	2	1
Lobular ..	+	3	0	0	3
Normal glomeruli	+	3	2	0	1
Uncomplicated cases	+	12	6	1	5
	−	4	2	1	1
Complicated cases	+	8	2	0	6
	−	5	1	3	1
Total cases	+	20	8	1	11
	−	9	3	4	2

[1] None of the four patients with progressive acute (proliferative) glomerulonephritis received cortisone.
[2] The minimal treatment period was three months, but most patients were treated continuously; "+", treated; "−", not treated.
[3] Minimal follow-up period after initial biopsy, two years; assessment based on serial histological study in 18 cases and on laboratory data in 11.

There were 15 cases of membranous glomerulo-nephritis; fluorescence occurred in four of six cases classified on the biopsy findings as diffuse membranous glomerulo-nephritis, but in none of nine cases of the membranous-proliferative type. Glomerular fluorescence occurred in single cases only of acute glomerulo-nephritis and nephropathy of pregnancy, and weakly in one of five cases of diabetic nephropathy.

Biopsy Findings in Other Renal Diseases

An additional 74 biopsies were performed on 72 patients in whom renal disease was suspected. The indications for biopsy and the histological findings are described below and in Table VII.

Minor Causes of Nephrotic Syndrome.—In five patients with nephropathy of pregnancy, proteinuria was first observed in the third trimester, and four of these developed the nephrotic syndrome. The biopsy showed normal renal tissue in two cases and minimal membranous-proliferative glomerulo-nephritis in three. Clinical resolution occurred in all five cases.

Amyloidosis was present in biopsy specimens from two single cases of septic osteomyelitis and mild rheumatoid arthritis. The nodular and diffuse deposits of amyloid stained with Congo red and toluidine blue and were situated in the glomerular capillary walls, causing capillary occlusion. A striking feature of these deposits was a positive reaction with the PAS stain, but it was weak compared with other types of membranous lesion.

In the one case of renal vein thrombosis there were slight focal basement membrane thickening and endothelial hyperplasia in the glomeruli, and pronounced interstitial fibrosis.

Acute Glomerulo-nephritis.—The indications for biopsy were the clinical suspicion of antecedent renal disease in two cases, polyarteritis nodosa in one, and non-resolution in three. The histological diagnosis was of decisive value in all six, being classical acute hæmorrhagic (Type I) glomerulo-nephritis in three cases and normal glomeruli subsequent to resolution in three.

TABLE VI

Results with Fluorescent Antibody Staining of Renal Biopsy in 71 Cases

Diagnosis	Number of Cases Studied	Number of Positive Results
Systemic lupus erythematosus: ..	15	
With glomerular lesions	11	8
Without glomerular lesions ..	4	0
Subacute glomerulo-nephritis: ..	21	
Acute progressive (proliferative)	4	1
Membranous diffuse	6	4
Membranous-proliferative ..	9	0
Lobular	2	0
Diabetic nephropathy	4	1 (weak)
Pregnancy nephropathy	5	1
Acute glomerulo-nephritis	5	0
Chronic nephritis	7	0
Hypertensive renal disease ..	5	0
Miscellaneous renal disease[1] ..	9	0

[1] Including cases of pyelonephritis, interstitial nephritis, sarcoidosis, scleroderma.

Chronic Nephritis.—Twelve cases presented clinically as "chronic nephritis" of indeterminate ætiology. The kidneys were radiologically small and functioned poorly. Biopsies were difficult to perform in this group, and multiple sessions were necessary in seven cases. The biopsy specimen was small and fragmented in three cases, and bleeding complicated three biopsies. Microscopically, the glomeruli showed varying degrees of hyalinization and sclerosis.

More than two-thirds of the glomeruli were sclerosed in six biopsy specimens, and in three others no clearly recognizable glomeruli were present. Renal scarring increased the likelihood of sampling error, and in three cases in which the biopsy specimen could be compared with autopsy sections, it was regarded as being non-representative. Thus, the diagnostic information obtained from renal biopsies in chronic nephritis was very limited.

Pyelonephritis.—This was associated in three cases with hypertension, in two cases with hyperchloræmic renal acidosis and renal calcification shown radiologically, and in single cases with pronounced fever, post-partum eclampsia and hypertension, gouty nephritis and prolonged septicæmia (Table VII). There was intraglomerular and periglomerular fibrosis in six biopsy specimens; the glomeruli were normal in the two cases of renal tubular acidosis. One patient had hypertension and proteinuria but no episodes of renal pain, and microscopic examination and culture of the urine repeatedly gave negative results, illustrating that "histological pyelonephritis" existed in the absence of all clinical features of the disease (Figure X).

Culture of the biopsy fragment was successful in only one of six suspected cases of pyelonephritis; a wider and more favourable experience with culture of renal biopsy material has been reported by Jackson *et alii* (1955).

Hypertension.—The biopsy established the diagnosis of benign essential hypertension in six cases and of malignant hypertension in three. Hypertensive vascular lesions were minimal in two cases, moderately advanced in four and severe in three. In three cases of severe hypertension (malignant in two), recent and moderate vascular damage encouraged sympathectomy being performed in two, whereas advanced vascular damage contraindicated surgery in one.

TABLE VII

Indications for Biopsy and Histological Findings in Renal Diseases Excluding Diabetes Mellitus, Systemic Lupus Erythematosus and Subacute Nephritis

Clinical Diagnosis	Number of Cases	Major Clinical Indication for Biopsy	Histological Findings
Nephrotic syndrome (minor causes):			
Pregnancy nephropathy ..	5	Albuminuria	Mild membranous-proliferative glomerulo-nephritis (3 cases); normal glomeruli (2 cases)
Amyloidosis	2	Albuminuria	Amyloidosis
Renal vein thrombosis (long-standing)	1	Albuminuria	Nephrosclerosis
Acute glomerulo-nephritis ..	6	Atypical presentation; assessment of resolution	Acute hæmorrhagic glomerulo-nephritis (3 cases) normal glomeruli (3 cases)
Chronic nephritis	12	Proteinuria, nitrogen retention, hypertension	Advanced scarring, glomerular obliteration and arteriosclerosis
Pyelonephritis	9	Hypertension, urinary infection	Interstitial fibrosis, glomerular crowding, tubular atrophy and dilatation, focal cellular infiltration
Hypertension	8	Assessment of hypertension	Nephrosclerosis, arteriosclerosis; arteriolonecrosis (3 cases)
Oliguria, anuria	7	Oliguria (4 cases); hepato-renal syndrome (3 cases)	Acute tubular necrosis (5 cases); severe interstitial nephritis (2 cases)
Liver disease:			
Hæmochromatosis	4	Assessment of renal function	Iron in collecting tubules; diabetic nephropathy (1 case)
Cirrhosis	2	Albuminuria	Glomerulo-sclerosis
Miscellaneous renal diseases:			
Sarcoidosis	2	Albuminuria (1 case); hypercalcæmia (1 case)	Patchy glomerular basement membrane thickening and adhesions (1 case); normal glomeruli and fibrosis and nephrocalcinosis (1 case)
Scleroderma	1	Hypertension	Slight focal glomerular basement membrane thickening; pronounced interstitial fibrosis
Familial nephritis	1	Albuminuria	Slight glomerular basement membrane thickening, tubular atrophy, fibrosis and cellular infiltration
Polycystic kidneys	1	Renal insufficiency	Normal glomeruli and tubules; biopsy non-contributory
Radiation nephritis	1	Hypertension	Glomerular hyalinization and sclerosis; tubular atrophy
Miscellaneous non-renal diseases[1] ..	14	Suspected renal disease	Normal renal tissue

[1] Cirrhosis of liver (6 cases), suspected carcinoma of kidney (2 cases), multiple myeloma, fibrous dysplasia of bone, rheumatoid arthritis, benzol poisoning, phenacetin addiction, suspected polyarteritis nodosa.

Acute Tubular Necrosis with Oliguria and Anuria. — Biopsy established a histological diagnosis in seven cases of acute oliguria. Sulphonamide anuria was differentiated from acute nephritis complicating tonsillitis in one; one patient, who presented with oliguria and hæmaturia after taking cantharides, had intact glomeruli, but necrosis of the convoluted tubules. Three patients with the hepato-renal syndrome associated with hyperbilirubinæmia (Martin and Taft, 1958) showed tubular necrosis.

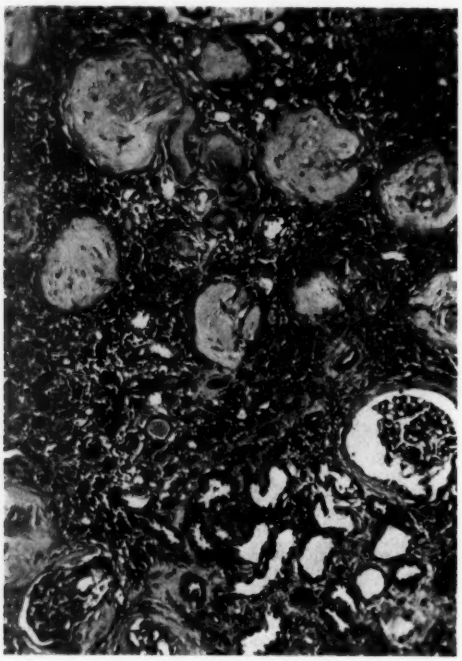

FIGURE X

A female, aged 45 years, with hypertension, renal insufficiency and normal urinary findings. Biopsy shows patchy glomerular sclerosis and crowding, vascular sclerosis, cellular infiltration and tubular atrophy: chronic pyelonephritis. (Low power. ×100)

In two subsequently fatal cases of acute oliguria, the biopsy revealed interstitial nephritis with diffuse infiltration of the parenchyma by leucocytes, but minimal glomerular and tubular damage.

Liver Disease.—In four patients with hæmochromatosis of the liver and diabetes mellitus, iron was present in the collecting tubules. One diabetic patient, who had required 40 to 60 units of insulin daily for 16 years, presented with systolic hypertension, microaneurysms in the retina and mild proteinuria; it was of interest that his renal biopsy demonstrated specific Kimmelstiel-Wilson lesions, as Lonergan and Robbins (1959) found that nephropathy rarely complicated diabetes due to hæmochromatosis.

Two patients with cirrhosis had protein and casts in the urine; biopsy showed a membranous-proliferative glomerulo-nephritis (see Martin and Taft, 1958), which resembled that described in cirrhosis by Patek *et alii* (1951) and by Fisher and Hellstrom (1959). Its pathogenesis is uncertain.

Non-Classified Cases. — Renal biopsy was performed on patients with sarcoidosis, scleroderma, familial nephritis, radiation nephritis and polycystic kidneys, the findings being described in Table VII. In 14 cases inflammatory, neoplastic or toxic renal disease was suspected clinically, but was excluded mainly on the basis of normal biopsy findings, and the subsequent course of these patients was in keeping with this.

DISCUSSION

Renal biopsy is of outstanding value in the assessment of the diagnosis, severity, natural history and effect of treatment of disease of the kidney. The information gained from renal biopsy studies could well represent the major advance in our knowledge of renal disease during the past decade.

The technique is more exacting and the risk of bleeding may be greater than with liver biopsy, particularly when the kidney is hard to localize because of œdema, fat or well-developed muscles, or when it is shrunken and fibrotic as in chronic nephritis. Our success rate of 68% for all biopsy attempts, whilst comparable with other figures cited by Brun and Raaschou (1958*a*), does not approach the 80% reported by Kark *et alii* (1958*a*). However, when repeated attempts were included, we obtained an adequate biopsy specimen in 200 (78%) of the 257 cases in this series.

Bleeding, which was commonest in elderly, hypertensive and azotæmic patients, and in those with fibrotic kidneys, complicated 4% of our biopsy attempts. This may have contributed to the death of two patients; one was gravely ill before the biopsy was performed, and in the other autopsy revealed extensive pathological changes in the heart and kidneys. Brun and Raaschou (1958*b*) list only three fatalities in over 1800 reported renal biopsies—possibly successes are more likely to be reported than fatalities. Although Brun and Raaschou

(1958*a*) discount hypertension and uræmia as predisposing to hæmorrhage, we use hypotensive drugs prior to biopsy on hypertensive patients (de Wardener and Hutt, 1956), and we reject patients with chronic nephritis in whom biopsy seems hazardous and of limited value.

Five glomeruli should be present in the biopsy specimen, although fewer may yield a diagnosis in diffuse renal disease. In post-mortem material, diagnostic assessment was not greatly enhanced when more than four glomeruli were included in the section (Kellow *et alii*, 1959). We found biopsy specimens to be representative and diagnostic, except from the scarred kidney of chronic nephritis, from which even autopsy sections may be indecisive. The correct diagnosis in our cases was often suspected beforehand, and biopsy was undertaken to assess the nature, degree and progress of renal damage; however, Parrish and Howe (1955) claimed that renal biopsy improved their diagnostic accuracy from 39% to 91%.

In diabetes, the correlation of nephropathy with hypertension, cardio-vascular disease and retinopathy was to be expected. All these manifestations might depend solely on the duration of the diabetes; however, nephropathy was occasionally demonstrable by biopsy soon after the onset of diabetic symptoms and well before clinical manifestations of renal disease appeared. The incidence of diabetic nephropathy did not correlate with the type of diabetes, "juvenile" or "senile", or with the degree of diabetic control; this is in agreement with the autopsy studies of Shea *et alii* (1959). Our findings thus suggest that some primary metabolic defect may determine the independent development of the diabetes and the renal lesions. However, Gellman *et alii* (1959), in a biopsy survey, found that diffuse glomerulosclerosis was "apparently more frequent" in juvenile diabetes, and that nodular and diffuse lesions were associated with an increased incidence of ketosis, indicative of poor diabetic control.

Progressing renal damage represents the major threat to life in systemic lupus erythematosus, and it is still debatable whether this is modified by treatment with cortisone. Our aim was to study the behaviour of histologically graded lupus nephritis in patients receiving continuous treatment with cortisone derivatives.

Our series of systemic lupus erythematosus comprised 17 patients with glomerulitis. In 15 cases the lesions were present in the initial biopsy specimen and presumably occurred early in the disease, whereas two of the six patients with initially normal biopsy findings subsequently developed nephritis despite treatment with cortisone. Of the 11 treated patients with "mild" and "moderate" glomerular lesions, sustained clinical and histological remission occurred in nine, but only one showed improvement over serial biopsies; in two cases the condition progressed, despite substitution of a high-dosage régime which caused pronounced hypercortisonism. Cortisone had adverse effects on patients with advanced lesions with hyalinization and fibrosis. Thus, prolonged treatment with cortisone derivatives usually arrests the progressing histological changes in early cases of lupus nephritis, but fails to produce healing of established glomerular lesions.

The clinical presentation of subacute, "protein losing" or "nephrotic" glomerulonephritis is fairly uniform; but the underlying histological lesions are variable and clinically unpredictable, emphasizing the necessity for biopsy to establish the complete diagnosis. In the four cases of proliferative glomerulo-nephritis, the course and microscopic features were those of progressive non-resolving acute nephritis, and streptococcal infection was implicated in all four. The membranous group was divided into diffuse membranous, membranous-proliferative and lobular subtypes (Allen, 1955; Kark *et alii*, (1958*b*), and although overlapping was inevitable, the histological criteria for these subtypes were usually sustained in serial biopsies.

The finding of normal glomeruli in some patients with the nephrotic syndrome is a feature of biopsy studies, being reported in 17 of 28 cases by Johnson and Reader (1959) and in 11 of 88 cases by Kark *et alii* (1958*b*), who classified such cases separately as "lipoid nephrosis", but were dubious about their differentiation from membranous glomerulonephritis (Folli *et alii*, 1958). A purely tubular cause for massive proteinuria may be discounted, on the basis of the electron microscopic findings of lesions involving the glomerular foot processes (Farquhar *et alii*, 1957), and because of the occasional transition, exemplified in one of our cases, from normal glomeruli to typical membranous glomerulo-nephritis. Therefore such cases were also classified as subacute nephritis.

Progress in subacute glomerulo-nephritis was assessed on clinical, biochemical and histological evidence; but greatest weight was placed on the last-mentioned, since apparent clinical remission was occasionally associated with histological deterioration. The rate of evolution of the glomerular lesions varied widely, indicating that subacute glomerulo-nephritis followed an

individual histological *tempo*, and underlining the difficulty of evaluating treatment.

Cortisone derivatives are of accepted value in subacute glomerulo-nephritis as diuretic agents (Derow, 1958; Johnson and Reader, 1959); but biopsy assessments of the long-term value of cortisone differ. Sharpe and Unger (1959) reported favourably, Blainey *et alii* (1960) reported improvement in patients with "minimal" and proliferative changes but not in patients with membranous lesions, whereas Baldwin and McCluskey (1959) were undecided. In our treated and untreated cases, which were of comparable severity, we could not demonstrate that prolonged treatment with cortisone conferred significant benefit in any subgroup; moreover, in certain cases the condition progressed relentlessly despite sustained high-dosage treatment. Further controlled studies, with serial biopsies, appear necessary before the long-term effectiveness of cortisone can be accepted. We would emphasize that our results apply particularly to adult patients; the childhood form of the disease may differ (Farquhar, 1960), and may show more biopsy specimens with normal glomeruli.

Our interest in the possible part played by autoantibodies in the pathogenesis of nephritis led us to apply rabbit anti-gamma globulin labelled with fluorescein to fragments of the biopsy specimen in order to recognize human gamma globulin bound to glomerular structures. The immunological significance of positive reactions in necropsy material was discussed by Mellors and Ortega (1956) and by Vazquez and Dixon (1958) in relation to renal disease in humans and nephrotoxic nephritis in animals.

In our experience a positive reaction was most frequently obtained in lupus nephritis, in keeping with the probable autoimmune nature of this disease. In membranous glomerulo-nephritis, a high proportion of positive reactions was obtained in the diffuse membranous type, but not in the membranous-proliferative or lobular types; this may have represented only a quantitative difference, in terms of the amount of protein in the basement membrane lesion, rather than reflecting a difference in pathogenesis. Freedman *et alii* (1960) obtained glomerular fluorescence in acute and membranous glomerulo-nephritis, in systemic lupus erythematosus, in scleroderma and in diabetic nephropathy, but were guarded as to the implications of their findings.

The fluorescing material in the glomeruli is probably equivalent to the proteinaceous material detectable in the basement membrane by electron microscopy (Movat and McGregor, 1959). It may represent either gamma globulin linked with a glomerular antigen, or the deposition in the glomerulus of an antigen-antibody complex combined elsewhere; if either is true, a fundamentally similar immunological process may determine the renal lesion in lupus nephritis, diffuse membranous glomerulo-nephritis, amyloidosis and experimental nephrotoxic nephritis.

Renal biopsy was also used to assess resolution in acute nephritis, to obtain histological and bacteriological confirmation of pyelonephritis, to differentiate renal from extrarenal causes of hypertension and to assess the degree of vascular damage in hypertension, to differentiate reversible from non-reversible causes of oliguria, and to establish the diagnosis of proteinuria of uncertain cause. Although in some cases of chronic nephritis it proved more useful to assess renal damage in terms of function than of structure, our experience indicated that renal biopsy was a procedure of great value in the study of renal disease; it will play a major role in the future.

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ACUTE HÆMOLYTIC ANÆMIA IN MEDITERRANEAN CHILDREN WITH GLUCOSE-6-PHOSPHATE DEHYDROGENASE-DEFICIENT ERYTHROCYTES[1]

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SUMMARY

A review has been made of certain clinical features of 20 episodes of acute hæmolytic anæmia sustained by 15 Australian children of Mediterranean racial extraction. When possible, metabolic studies were made on the erythrocytes of these 15 children.

Deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD) was found in the erythrocytes of the 13 children tested. Marked instability of reduced glutathione (GSH) and susceptibility to Heinz-body formation on incubation with acetyl phenylhydrazine were demonstrated in the G6PD-deficient erythrocytes of 10 of these 13 children.

The outstanding feature of the clinical study was the high incidence of males, of springtime illness, and of exposure to *Vicia fava*. Ingestion of broad beans closely preceded the onset of nine hæmolytic episodes, and five additional episodes occurred in ambulant males in spring. The erythrocytes of these children were characterized by very marked degrees of G6PD deficiency, GSH instability and susceptibility to Heinz-body formation.

A second striking feature was the demonstration of erythrocyte G6PD deficiency in three infants with severe neonatal hyperbilirubinæmia. One infant had received 2 mg. of a vitamin K analogue, another may have ingested phenacetin or acetylsalicylic acid in maternal breast milk, and in the third case no toxic agent was found. One infant subsequently sustained an attack of favism.

Erythrocyte G6PD deficiency was also found in one child who developed acute hæmolytic anæmia with acute glomerulonephritis, and in another who sustained a mild hæmolytic episode in association with intestinal salmonellosis.

The relationship between neonatal jaundice, favism and drug-induced hæmolysis has been discussed in terms of the mode of genetic transmission of G6PD deficiency, with reference to the protection of potentially susceptible individuals from potentially hæmolytic agents.

DURING the past five years, the problem of acute hæmolytic anæmia in Australian children of Mediterranean extraction has gained increasing attention. In a review of the patients with acquired hæmolytic anæmia admitted to the Royal Alexandra Hospital for Children during the period 1954 to 1957 (Harley and Dods, 1959), four of the eight children were found to be males of Mediterranean extraction; in these four cases the response to the Coombs test was negative. Ingestion of the Australian broad bean, *Vicia fava*, closely preceded the onset of hæmolysis in one of these males, whose erythrocytes were subsequently shown to be relatively deficient in reduced glutathione (GSH). Isolated reports of favism in Mediterranean children have also appeared in the Australian medical literature (Brooks *et alii*, 1958; Moore, 1958; McCutcheon, 1960). Recently, attention has been directed to two infants with erythrocytes deficient in the enzyme glucose-6-phosphate dehydrogenase (G6PD), who developed severe hyperbilirubinæmia in the neonatal period (Lee *et alii*, 1961).

The object of this communication is to review six additional Mediterranean children with acute hæmolytic anæmia as well as the cases already reported in the Australian literature, and to present the results of certain metabolic studies on the erythrocytes of the children under review.

MATERIALS AND METHODS

A review has been made of certain clinical features of the 11 episodes of acute hæmolytic anæmia in Mediterranean children already reported in the Australian medical literature (Harley and Dods, 1957, 1959; Robertson, 1957; Brooks *et alii*, 1958; Moore, 1958; McCutcheon, 1960; Lee *et alii*, 1961), and of

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nine previously unreported examples of such episodes. The combination of transient anæmia, reticulocytosis, spherocytosis and hyperbilirubinæmia has been assumed to justify a diagnosis of acute hæmolytic anæmia.

The relevant clinical features were taken from the published case reports. The nine unreported episodes of acute hæmolysis occurred in six children who attended the Royal Alexandra Hospital for Children during the periods 1950 to 1953 or 1958 to 1960. The clinical features were taken from the hospital case records, summaries of which are presented below. In this publication, Case numbers I to IX denote the cases already reported, and Case numbers X to XV the previously unreported cases.

When possible, the following studies were made on the erythrocytes of the 15 children under review. Erythrocyte G6PD activity was assayed by the method of Zinkham (1959). The GSH content of erythrocytes was determined by the method of Grunert and Phillips (1951), as modified by Beutler (1957), and the GSH stability test was done by the method of Beutler (1957). The Heinz body incubation test was performed by the method of Beutler *et alii* (1955). With the exception of the results in the case described by McCutcheon (1960), the results presented were all obtained in this laboratory. Unless otherwise stated, these investigations were made at least three months after recovery from the acute hæmolytic episode. Some or all of these studies were also made on the erythrocytes of 19 normal Anglo-Saxon children, aged from two to 12 years, and of 10 normal Mediterranean children, aged from one to 12 years.

REPORTS OF CASES

CASE X.—L.P., a male child of North Italian extraction, aged 10 years, was admitted to hospital on October 24, 1960, with a history of pallor, lassitude, abdominal pain and dark-red urine for three days, after eating fresh broad beans about 10 days previously. On examination of the patient, pallor and jaundice were noted, and the spleen was not palpable. The results of laboratory investigations were as follows. The hæmoglobin value was 4·8 grammes per 100 ml., and the erythrocytes numbered 1,380,000 per cubic millimetre; reticulocytes comprised 20% of the erythrocytes; occasional spherocytes were present in the peripheral blood. The total serum bilirubin content was 2·4 mg. per 100 ml., and methæmalbumin was detected in the plasma. The response to the direct Coombs test was negative.

The child's condition improved rapidly without blood transfusion, and he was discharged from hospital four days after admission. Three months later he appeared well, the hæmoglobin value was 12·1 grammes per 100 ml., and no abnormality was detected in a smear of peripheral blood.

CASE XI.—V.G., a male child, aged two years, of Sicilian extraction, was admitted to hospital on October 10, 1960, with a history of vomiting, jaundice and dark urine of one day's duration following the ingestion of cooked broad beans on the previous day. On examination, the child was pale and jaundiced, and the spleen was not palpable. On laboratory investigation, the hæmoglobin value was 4·0 grammes per 100 ml., reticulocytes comprised 13% of the erythrocytes, and numerous spherocytes were present in the peripheral blood. The response to the direct Coombs test was negative. The child improved rapidly after blood transfusion, and when he was examined four months later seemed very well; his hæmoglobin value was 13·0 grammes per 100 ml., and no abnormality was detected in a smear of peripheral blood.

CASE XII.—C.G., a male child of Corinthian extraction, aged three years, was admitted to hospital on November 17, 1958, with a history of malaise and jaundice of 24 hours' duration, which had commenced almost immediately after eating fresh broad beans. On examination, the child was pale and jaundiced and the spleen was not palpable. The results of laboratory investigations were as follows. The hæmoglobin value was 3·9 grammes per 100 ml., and the erythrocytes numbered 1,390,000 per cubic millimetre; reticulocytes comprised 7% of the erythrocytes; erythroblasts numbered 7500 per cubic millimetre; numerous spherocytes were present in a smear of peripheral blood. The total serum bilirubin content was 9·2 mg. per 100 ml., and spectroscopic examination revealed the presence of methæmalbumin in the plasma and oxyhæmoglobin in the urine. The response to the direct Coombs test was positive in titres ranging from 1 : 4 to 1 : 32.

After blood transfusion, marked improvement was noted, the response to the direct Coombs test became negative, and the child was discharged from hospital. Two years later, the child appeared well; his hæmoglobin value was 12·1 grammes per 100 ml., and no abnormality was detected in a smear of peripheral blood.

These three male children thus represent further cases of favism. Each sustained an indisputable episode of excessive hæmolysis soon after the springtime ingestion of the beans of *Vicia fava*. Recovery from the illness was rapid in each, and occurred without blood transfusion in one of these cases. In one child, the response to the direct Coombs test was transiently positive during the hæmolytic episode.

CASE XIII.—J.N., a male child of Greek extraction, from the island of Lemnos, was admitted to hospital on September 30, 1953, at the age of three years, with a history of pallor, jaundice and abdominal pain of three days' duration. On examination, he was pale and jaundiced and the spleen was not palpable. The results of investigations were as follows. The hæmoglobin value was 4·0 grammes per 100 ml., erythrocytes numbered 1,300,000 per cubic millimetre, reticulocytes comprised 25% of the erythrocytes, and occasional spherocytes and erythroblasts were present in the peripheral blood. The response to the direct Coombs test was negative. The child's condition improved after blood transfusion, and he was discharged from hospital on October 14. The parents could suggest no noxious agents which might have precipitated the illness.

The child was readmitted to hospital on January 10, 1955, at the age of four years, with a history of pallor and dark urine of three days' duration. On examination, he was pale, but not jaundiced. The hæmoglobin value was 9·00 grammes per 100 ml., erythrocytes numbered 3,200,000 per cubic millimetre, and reticulocytes comprised 5% of the erythrocytes. The total serum bilirubin content was less than 0·5 mg. per 100 ml. On routine culture of stool a moderate growth of Salmonella sp., not *Salmonella typhi*, was found, and the child was discharged from hospital. When he was examined six months later, he appeared well, the hæmoglobin value was 13·4 grammes per 100 ml., and no abnormality was detected in a smear of peripheral blood.

CASE XIV.—S.D., a male child of Greek extraction, was first admitted to hospital on January 5, 1952, at the age of 10 months, with a history of pallor, jaundice and dark urine of two days' duration. On examination, he was pale and jaundiced, and the spleen was palpable 2 cm. below the left costal margin. The results of laboratory investigation were as follows. The hæmoglobin value was 2·9 grammes per 100 ml., and erythrocytes numbered 910,000 per cubic millimetre; reticulocytes comprised 10% of the erythrocytes; erythroblasts numbered 1200 per cubic millimetre; occasional spherocytes were present in the peripheral blood. The response to the direct Coombs test was negative. After blood transfusion, the child's condition improved rapidly, and he was discharged from hospital on February 2.

The child was readmitted to hospital on October 21, 1952, at the age of 19 months, with a similar history of pallor and jaundice of several days' duration. On examination of the patient, pallor and jaundice were noted. On laboratory investigation, the hæmoglobin value was 6·3 grammes per 100 ml., erythrocytes numbered 2,200,000 per cubic millimetre, and numerous spherocytes and polychromatic cells were present in the peripheral blood. After blood transfusion, marked improvement was again noted and the child was discharged from hospital on October 24.

He was readmitted for the third time on November 29, 1953, with a history of abdominal pain and vomiting of two days' duration. On examination, he was pale but not jaundiced. Laboratory investigations gave the following results. The hæmoglobin value was 9·5 grammes per 100 ml., erythrocytes numbered 2,900,000 per cubic millimetre, reticulocytes comprised 17% of the erythrocytes, and numerous spherocytes were present in the peripheral blood. The total serum bilirubin content was 0·8 mg. per 100 ml. The response to the direct Coombs test was negative. The child improved rapidly after blood transfusion, and was discharged from hospital on December 9. When he was examined on April 7, 1961, he seemed very well; his hæmoglobin value was 14·4 grammes per 100 ml., and no abnormality was detected in a smear of peripheral blood.

The child's parents had immigrated from Cyprus, where his maternal uncle had died from "anæmia" in childhood. The parents were unaware of exposure of the child to any toxic substance, but mentioned that he was still receiving breast milk at the time of the first illness. The mother was unable to recall the ingestion of any toxic agent while breast-feeding the infant.

In all, these two male children sustained five hæmolytic episodes. Although neither spherocytosis nor hyperbilirubinæmia was demonstrated in the second episode in Case XIII, the combination of pallor and dark urine of sudden onset with transient anæmia and reticulocytosis appeared to justify a diagnosis of acute but mild hæmolysis. Intestinal salmonellosis coincided with this second mild hæmolytic episode. The child in Case XIV first became ill

TABLE I

Certain Clinical Features of the 20 Hæmolytic Episodes Sustained by the 15 Children under Review

Case Number and Patient's Designation	Reference	Sex	Racial Extraction	Age of Onset	Month of Onset	Ætiological Agent	Transfusion	Outcome of Illness
I : P.P.	Harley and Dods (1957, 1959)	M.	Greek (Imbros)	7 years	October	*Vicia fava*	+	Recovery
II : V.V.	Robertson (1957); Harley and Dods (1959)	M.	Italian (north)	10 months	April	? Acute glomerulonephritis	+	Recovery
III : —	Brooks *et alii* (1958)	F.	Greek (Dodecanese Is.)	3 years	October	*Vicia fava*	+	Recovery
				3 years	March	*Vicia fava*; ? sulphonamide	–	Recovery
IV : —	Moore (1958)	M.	Italian	5 years	November	*Vicia fava*	+	Recovery
V : M.V.	Harley and Dods (1959)	M.	Greek; Syrian; Egyptian	3 years	November	Unknown	+	Recovery
VI : S.C.	Harley and Dods (1959)	M.	Italian (south)	3 years	November	Unknown	+	Recovery
VII : —	McCutcheon (1960)	M.	Italian	2 years	Not recorded	*Vicia fava*	+	Recovery
VIII : L.L.	Lee *et alii* (1961)	M.	Italian (south)	3 weeks	March	? Phenacetin; ? acetylsalicylic acid	–	Recovery
				7 months	October	*Vicia fava*; ? chloramphenicol	–	Recovery
IX : M.A.	Lee *et alii* (1961)	F.	Greek (Cephalonia)	3 days	March	Vitamin K analogue (menaphthone sodium bisulphite)	–	Recovery
X : L.P.	——	M.	Italian (north)	10 years	October	*Vicia fava*	–	Recovery
XI : V.G.	——	M.	Italian (Sicily)	2 years	October	*Vicia fava*	+	Recovery
XII : C.G.	——	M.	Greek (Corinth)	3 years	November	*Vicia fava*	+	Recovery
XIII : J.N.	——	M.	Greek (Lemnos)	3 years	September	Unknown	+	Recovery
				4 years	January	? Salmonella	–	Recovery
XIV : S.D.	——	M.	Greek (Cyprus)	10 months	January	Unknown	+	Recovery
				1 year	October	Unknown	+	Recovery
				2 years	November	Unknown	+	Recovery
XV : W.A.	——	M.	Greek (Dodecanese Is.)	10 days	October	Unknown	+	Recovery

while receiving breast milk from the mother, who was unaware of coincidental ingestion of any toxic agent. Although a history of exposure to *Vicia fava* was not elicited, each of the three remaining episodes sustained by these children took place in the springtime.

CASE XV.—W.A., a male infant of Greek parents, from the Dodecanese Islands, was delivered normally on September 20, 1960. Jaundice was evident at the age of 24 hours, but became more pronounced on the third day, when the total serum bilirubin content was 12·1 mg. per 100 ml. The jaundice then decreased, and the child was discharged from the obstetric hospital on the eighth day. At the age of 10 days, however, he suddenly became severely jaundiced and was admitted to the Royal Alexandra Hospital for Children. On examination, he was pale and very jaundiced, but the spleen was not palpable. The results of laboratory investigations were as follows. The hæmoglobin value was 10 grammes per 100 ml.; reticulocytes comprised 9% of the erythrocytes; numerous spherocytes were detected in the peripheral blood. The total serum bilirubin content was 33 mg. per 100 ml., and the conjugated serum bilirubin content was 0·4 mg. per 100 ml. Oxyhæmoglobin was detected on spectroscopic examination of the urine. The mother's blood group was O, Rh(D) positive, and the infant's blood group A, Rh(D) positive. The response to the direct Coombs test was negative in the infant, and no hæmolysis of the infant's cells was noted after incubation with the mother's serum at 37° C. for one hour. No evidence of incomplete antibodies was detected in the infant's serum against the infant's papainized erythrocytes.

On the next day the hæmoglobin value was 6·0 grammes per 100 ml., and the total serum bilirubin content was 20 mg. per 100 ml. A transfusion of 100 ml. of whole blood was given. After the transfusion, the hæmoglobin value slowly decreased to a value of 8·4 grammes per 100 ml. on the twenty-second day of life, when a second transfusion of 100 ml. of blood was given. After this second transfusion his condition improved, and he was discharged from hospital on October 15, 1960. Three months later the infant was well, the hæmoglobin value was 13·0 grammes per 100 ml., and no abnormality was detected in a smear of peripheral blood.

This new-born infant also suffered from acute hæmolytic anæmia. Notable features were the severe degree of hyperbilirubinæmia, the absence of evidence of isoimmunization, and the failure to incriminate any toxic agent. Recovery from the illness occurred after two blood transfusions.

RESULTS

Clinical Features

The relevant clinical features are summarized in Table I. Three children sustained two, and one child three, episodes of excessive hæmolysis, so that a total of 20 episodes occurred in the 15 children under review. Of these 15 children, 13 were males and two females. The racial background was Italian in seven children, with one from Sicily and none from Sardinia, and Greek in eight children, with two from the Dodecanese Islands, one from each of the islands of Imbros, Lemnos, Cyprus and Cephalonia; one child was of mixed Greek, Syrian and Egyptian extraction.

The age of onset and seasonal incidence are represented diagrammatically in Figure I. The age of onset was less than four weeks in three, less than one year in six, and less than four years in 16 of the 20 episodes, but ranged to 10 years in the remaining four children. The seasonal incidence was such that of the 20

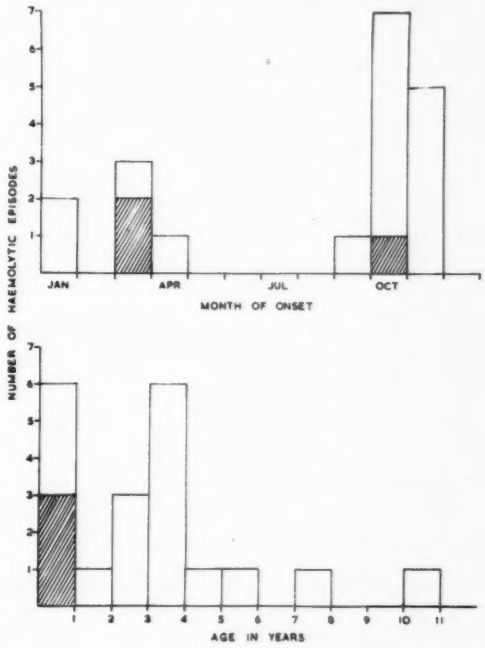

FIGURE I

Seasonal incidence and age of onset of the 20 episodes of excessive hæmolysis under review. The shaded areas represent infants aged less than four weeks

hæmolytic episodes, 13 occurred during the months of September, October or November, and thus during the Australian springtime.

Vicia fava was the most common ætiological agent (Table I). Broad bean ingestion closely preceded nine hæmolytic episodes in eight children. More remotely related to two attacks of favism were undiagnosed respiratory tract infections, for which one child received sulphonamide and another received chloramphenicol. Possible ætiological factors in two new-born infants included a vitamin K analogue (menaphthone sodium bisulphite), phenacetin and acetylsalicylic acid. Acute hæmolytic anæmia was associated in one infant with acute glomerulo-

nephritis, and in another child with intestinal salmonellosis. Five of the remaining hæmolytic episodes, in which the ætiological agent was unknown, occurred in ambulant males in springtime.

A blood transfusion was given in 14, and recovery without transfusion occurred in six hæmolytic episodes. Of the three new-born infants, one received simple blood transfusion

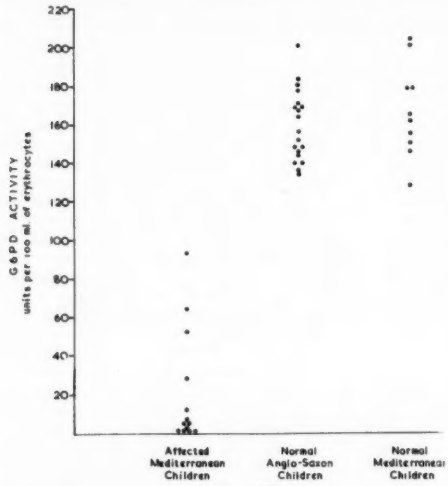

FIGURE II

Glucose-6-phosphate dehydrogenase (G6PD) activity of the erythrocytes of 13 of the 15 children under review, of 19 normal Anglo-Saxon children and of 10 normal Mediterranean children

and each recovered completely, without evidence of kernicterus, without exchange transfusion. In each of the 12 older children, complete recovery also occurred.

Metabolic Studies on Erythrocytes

The G6PD activity was assayed in the erythrocytes of 13 of the 15 affected children, of 19 normal Anglo-Saxon children and of 10 normal Mediterranean children. From Figure II the erythrocyte G6PD activity in each of the 13 affected children may be seen to be well below the range of that of either group of normal children.

The GSH stability test was performed on the G6PD-deficient erythrocytes of 12 of these 13 children, and on the erythrocytes of seven children with normal G6PD activity (Figure III). When normal erythrocytes were incubated with acetyl phenylhydrazine, a slight fall in the concentration of GSH occurred. With the G6PD-deficient erythrocytes of 10 children, however, the initial GSH content tended to be lower and the fall in GSH concentration much more pronounced. Alternatively, the G6PD-deficient erythrocytes from two children responded normally to the GSH stability test.

The Heinz body incubation test was also done on the G6PD-deficient erythrocytes of 12 children, and on the erythrocytes of nine children with normal G6PD activity (Figure III). After incubation under standard conditions with acetyl phenylhydrazine, from 5% to 18% of the erythrocytes from the normal children contained more than five Heinz bodies. In contrast, from 50% to 98% of the G6PD-deficient erythrocytes from 11 of the 12 children contained more than five Heinz bodies. The result was more equivocal in one of the 12 children, with 28% of the erythrocytes containing more than five Heinz bodies.

The results of G6PD assay, of the GSH stability test and of the Heinz-body incubation test in the affected children are correlated in Table II. Thus in Case I, Cases V to VIII and Cases X to XIV, marked G6PD deficiency

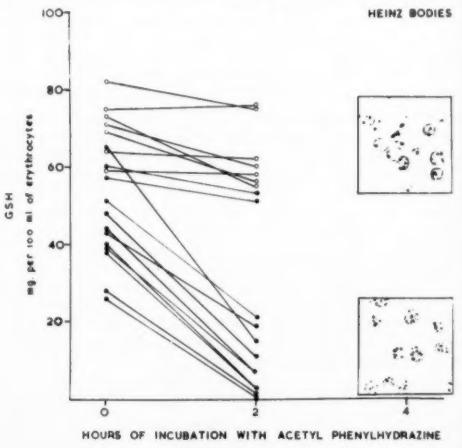

FIGURE III

Results of the glutathione (GSH) stability test on 12 children with G6PD-deficient erythrocytes and seven children with normal erythrocyte G6PD activity. Inset are typical results of the Heinz body incubation test, with normal erythrocytes (above—open circles) and with G6PD-deficient erythrocytes (below—solid circles)

was associated with marked GSH instability and susceptibility to Heinz-body formation. In Case II, moderate G6PD deficiency was found with normal GSH stability and an equivocal result to the Heinz-body incubation test. In Cases IX and XV, G6PD activity was assayed at the time of the acute illness during the neonatal period. The range for normal

new-born infants is 215 to 410 units per 100 ml. of erythrocytes (Zinkham, 1959). The G6PD activity in these two infants was thus well below the normal neonatal range. Normal GSH stability was associated with G6PD deficiency and marked susceptibility to Heinz-body formation during the acute illness of the new-born infant in Case IX.

Correlation of Clinical Findings with Erythrocyte Studies

The cases appear to fall into two main groups. The larger comprises children, usually males and usually ambulant, whose clear-cut episode of acute hæmolytic anæmia either was directly related to ingestion of *Vicia fava*, or else occurred in the springtime without known exposure to broad beans. The erythrocytes from these children were characterized by very marked degrees of G6PD deficiency, GSH instability and susceptibility to Heinz-body formation.

TABLE II

Results of Metabolic Investigation of the Erythrocytes of the Children under Review

Case	Units of G6PD per 100 ml. of Erythrocytes	Milligrammes of GSH per 100 ml. of Erythrocytes		Percentage of Erythrocytes with More than Five Heinz Bodies after Incubation with Acetyl Phenylhydrazine
		Before Incubation	After Incubation	
I	0	28	2	92
II	52	57	51	28
III	—[2]	—	—	—
IV	—	—	—	—
V	5	26	1	96
VI	0	40	4	98
VII	6	48	11	50
VIII	5	65	15	92
IX	93[1]	60[1]	53[1]	93[1]
X	28	43	19	89
XI	12	44	7	86
XII	3	51	21	88
XIII	0	38	4	90
XIV	1	39	7	95
XV	64[1]	—	—	—

[1] Investigation performed during acute hæmolytic episode.
[2] "—", investigation not performed at any stage.

The second and smaller group is made up of three infants aged less than four weeks, in whom pronounced hyperbilirubinæmia was associated with erythrocyte G6PD deficiency. The male infant in Case VIII developed acute hæmolytic anæmia with hyperbilirubinæmia at the age of 23 days, after possible exposure to phenacetin and acetylsalicylic acid in the breast milk of the mother, and again at the age of seven months, after eating broad beans. Severe G6PD deficiency and GSH instability were found after recovery from this second illness. The female infant in Case IX, at the age of eight days, exhibited severe hyperbilirubinæmia with spherocytosis following the injection of 2 mg. of a vitamin K analogue. On the eleventh day, G6PD deficiency was found with normal GSH stability and pronounced susceptibility to Heinz-body formation. Although neither anæmia nor reticulocytosis was evident in this infant, the combination of transient hyperbilirubinæmia and spherocytosis with erythrocyte G6PD deficiency was taken to justify the assumption of an acute episode of excessive hæmolysis. The male infant in Case XV developed acute hæmolytic anæmia with severe hyperbilirubinæmia at the age of 10 days, but no toxic agent was found. When assayed on the twenty-first day of life, the erythrocyte G6PD activity was found to be well below the range for either normal children or normal new-born infants.

There remain two children with G6PD-deficient erythrocytes and less typical clinical features. The infant in Case II sustained an episode of acute hæmolytic anæmia associated with acute glomerulo-nephritis at the age of 10 months. During the acute illness, on one occasion, the blood urea content was elevated to 66 mg. per 100 ml. Subsequently, a moderate degree of G6PD deficiency was found, with normal GSH stability and equivocal susceptibility to Heinz-body formation. In contrast, pronounced erythrocyte defects were present in the child in Case XIII, who suffered simultaneously from a mild hæmolytic episode and intestinal salmonellosis.

DISCUSSION

The outstanding feature of this clinical review of 20 acute hæmolytic episodes in 15 Mediterranean children is the high incidence of males, of springtime illness and of known exposure to *Vicia fava*. Favism is seasonal, most cases occurring in spring when the beans are fresh and the pollen of the blossoms is in the air (Crosby, 1956). Of the nine attacks of favism in the present review, eight occurred in spring and one followed the ingestion of dried broad beans during March. The possibility of favism was also suggested by the springtime onset of five hæmolytic episodes in ambulant males. While the paucity of other ætiological agents in ambulant children is unexplained, the relative frequency of favism in Australia may well be related to the favourable effect of the Australian coastal climate on the cultivation of broad beans.

The erythrocytes of subjects who are unduly sensitive to the hæmolytic effect of primaquine, *Vicia fava* and other toxic agents are characterized by undue susceptibility to Heinz-body production when incubated with acetyl phenylhydrazine (Beutler *et alii*, 1955), by inability

to protect their GSH content from oxidation when incubated with acetyl phenylhydrazine (Beutler, 1957), and by deficiency in the enzyme G6PD (Carson *et alii*, 1956). A high incidence of these defects was demonstrated in the children in this series. In particular, severe defects were found in those ambulant males who had suffered from favism, or from acute hæmolysis in springtime without known exposure to *Vicia fava*. The association of normal GSH stability with G6PD deficiency during the acute illness of the new-born infant in Case IX has been discussed previously (Lee *et alii*, 1961). The drugs which are known to cause hæmolysis in sensitive subjects include sulphonamide, phenacetin, acetylsalicylic acid, certain vitamin K derivatives (de Gruchy, 1960), and chloramphenicol (Brunetti *et alii*, 1959).

The high male incidence may be related to the mode of genetic transmission of G6PD deficiency and GSH instability. The gene for GSH instability is thought to be sex-linked with variable expressivity (Childs *et alii*, 1958), so that the male hemizygote and female homozygote would cause marked GSH instability, while the female heterozygote might show all degrees of expression from marked loss of stability to apparent normality of the erythrocytes. A positive correlation between the GSH stability test and G6PD deficiency has been reported by Gross and Marks (1958), and marked degrees of these defects are more commonly found among males than among females (Alving *et alii*, 1958; Zinkham, 1959). Alving *et alii* (1958) have shown primaquine-induced hæmolysis in a recipient of erythrocytes from females with normal or intermediate GSH stability, and have pointed out that in-vivo hæmolysis is a different phenotype from that shown by in-vitro testing. However, the preponderance in the present study of males with severe defects does suggest a close relationship between the degrees of metabolic defect *in vitro* and of hæmolysis *in vivo*.

The slight Greek preponderance in this series does not reflect the present 3 : 1 ratio of Italians to Greeks in Australia ("Australian Immigration Quarterly Statistical Bulletin", 1960). Noteworthy also is the extraction of five children from the eastern chain of Greek islands, and the absence of evidence of ancestral derivation from the island of Sardinia. The incidence of favism in Sardinia is said to be the highest in the world, and Crosby (1956) has suggested that the hereditary susceptibility to favism may have originated in Sardinia and been disseminated in the days when Phoenicians, Greeks, Carthaginians and Romans took Sards for slaves. After studying the incidence of erythrocyte defects in various populations, Szeinberg and Sheba (1959) suggested alternatively that the gene defect originated in Israel and was disseminated to the Mediterranean islands when the Canaanites and Hebrews set up the various outposts of the Punic maritime empire. Such an origin would also explain the high defect incidence among Kurdistan and Iraqi Jews. While no conclusion can be drawn from the small group presented here, these observations emphasize the value of such methods of investigation as a tool for anthropological screening of migratory populations from the Mediterranean shore.

The second striking feature of this review was the association in three new-born infants of severe hyperbilirubinæmia with G6PD deficiency. The significance of G6PD deficiency in the development of severe neonatal jaundice, often with kernicterus, has been stressed in recent reports from both Singapore (Smith and Vella, 1960) and Athens (Doxiadis *et alii*, 1961). Although no toxic agent was found in more than half the cases described by Doxiadis *et alii*, many infants were exposed to vitamin K and other toxic substances, and the importance was stressed of protecting new-born infants of susceptible racial groups from such hæmolytic agents. Of the three infants presented here, one had received 2 mg. of a vitamin K analogue, one may have been exposed to phenacetin or acetylsalicylic acid in the breast milk of the mother, and in one case no toxic agent was found. The subsequent development of favism in one of these infants is relevant to the suggestion of Doxiadis *et alii* that particular attention be paid to protecting the new-born infants of families with a history of favism, drug-induced hæmolysis or neonatal jaundice. Lee *et alii* (1961) have stressed the hazard to infants of ingesting products of *Vicia fava* and other toxic substances in the breast milk of the mother.

Questions of ætiological relationship arise in the child with G6PD-deficient erythrocytes, in whom acute hæmolysis accompanied a disease indistinguishable from acute glomerulo-nephritis. The association of acute hæmolytic anæmia with acute glomerulo-nephritis has been discussed by Hensley (1952) and by Robertson (1957). In the present case, the possibility must be considered that the same unrecognized ætiological agent or disease process produced both glomerulo-nephritis and hæmolytic anæmia. Alternatively, it is tempting to postulate that degrees of nitrogen retention which do not affect normal cells may produce excessive hæmolysis in G6PD-deficient erythrocytes. The association of normal GSH stability with G6PD deficiency

in this child is unexplained, but has been reported previously (Larizza *et alii*, 1958; Zinkham, 1959), and does not deny the presence of metabolic abnormality in the erythrocytes.

The relationship of bacterial and viral infections to in-vivo hæmolysis of G6PD-deficient erythrocytes is also obscure. The child in Case XIII suffered simultaneously from mild hæmolysis and intestinal salmonellosis, and two other children sustained undiagnosed respiratory tract infections several weeks before indisputable attacks of favism. Cases of hæmolysis have been encountered in the non-Ashkenazic population of Israel following typhoid fever, viral infections and even "Asian 'flu" (Szeinberg, Sheba and Adam, 1958). Acute hæmolytic anæmia associated with *Salmonella* (Dublin strain) septicæmia has also been reported (Davidson and Fullerton, 1938) in an adult female of unrecorded racial extraction. Despite the tenuous link between infection and hæmolysis in the cases presented here, the stress of infection may well have been at least one factor in precipitating the hæmolysis of defective erythrocytes.

The complete recovery from six hæmolytic episodes without blood transfusion, and from three episodes of neonatal jaundice without exchange transfusion, must be assessed in terms of the potential mortality of favism and of the high incidence of kernicterus following neonatal jaundice due to G6PD deficiency. Crosby (1956) reported that 40 to 50 deaths from favism occur annually in Sardinia, usually in children aged less than six years, and that early and adequate transfusion produced a noticeable transformation in moribund children. Doxiadis *et alii* (1961) stressed the need, in the care of G6PD-deficient infants with neonatal jaundice, for close observation of the serum bilirubin levels and exchange transfusion when dangerous levels are reached. The complete recovery of each child in the present series serves to emphasize the importance of critical evaluation of the need for simple transfusion throughout the hæmolytic episode, and of exchange transfusion in the presence of neonatal jaundice.

Finally, the question arises as to the way in which these erythrocyte defects produce increased susceptibility to drug-induced hæmolysis. Certain drugs damage the cell by destroying hæmoglobin and producing Heinz bodies, or by an independent effect which leads to increased osmotic fragility, or by both mechanisms (Harley and Mauer, 1961). Erythrocyte GSH appears to play an essential part both in protecting hæmoglobin from oxidative destruction (Mills, 1957), and in maintaining cell integrity (Fegler, 1952). Glutathione is maintained in its reduced form by the oxidation of glucose-6-phosphate via the pentose phosphate pathway, which utilizes the enzyme G6PD. The inability of the G6PD-deficient erythrocyte to protect GSH when challenged with certain drugs may thus lead to increased susceptibility to hæmoglobin destruction, to Heinz-body production, to decreased osmotic resistance and to loss of cell integrity.

Although the effects of some chemical substances on defective and normal cells are thus to some extent predictable, certain aspects of the mode of action of *Vicia fava* have, until recently, been obscure. Metabolic defects are consistently found in the erythrocytes of patients with a past history of favism (Szeinberg, Asher and Sheba, 1958), and a selective effect on the GSH stability of sensitive erythrocytes was recently shown when normal and sensitive cells were incubated with extracts from the beans, pollen and pistils of *Vicia fava* (Bowman and Walker, 1961). However, some subjects with defective erythrocytes are known to have eaten fava beans with impunity (Szeinberg, Sheba and Adam, 1958). This impunity may be related to the observation of Roth and Frumin (1960) that normal plasma or serum protected erythrocytes from the sensitizing ability of bean extract, but that plasma or serum from a patient with favism did not have this effect. These authors suggested that a serum deficiency renders persons with G6PD-deficient erythrocytes susceptible to favism. The combination of immunological and metabolic factors in the pathogenesis of favism was illustrated in Case XII of the present series, by the demonstration of a transiently positive response to the direct Coombs test during the acute episode, and of marked G6PD deficiency and GSH instability after the cessation of excessive hæmolysis. The low age incidence in the children presented also suggests the possibility of an acquired immunity to *Vicia fava*.

These observations thus illustrate the varied clinical manifestations of erythrocyte G6PD deficiency. New-born infants are prone to severe hyperbilirubinæmia and kernicterus, both with and without known exposure to hæmolytic agents. Older children are susceptible to the hæmolytic effect of a wide range of chemical substances, and certain of these children may develop acute hæmolysis on exposure to the beans, pollen or pistils of *Vicia fava*. Adults also may be susceptible, and the absence of Australian reports of drug-induced hæmolysis in G6PD-deficient adults is unexplained. The racial incidence of the defect directs particular attention in this country to the members of

Mediterranean racial groups. Furthermore, the defect is congenital and genetically determined, so that subjects with a personal or family history of neonatal jaundice, drug-induced hæmolysis or favism are potentially susceptible. To such susceptible individuals, many chemical and vegetable substances present hazards from which protection is essential from the perinatal period into childhood and throughout adult life.

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ARTIFICIAL COUGHING FOR PATIENTS WITH RESPIRATORY PARALYSIS[1] [3]

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SUMMARY

A simple machine by which exsufflation with negative pressure (E.W.N.P.) can be performed to provide an artificial cough is described. Artificial coughing by this means allowed the control of lung complications in 20 patients with respiratory paralysis in whom retained bronchial secretions were an important problem. Massive collapse was relieved on eight occasions in seven patients. Prophylactic coughing makes possible the removal of a tracheotomy tube from patients with negligible respiratory muscle power.

E.W.N.P. produces changes essentially similar to those occurring during normal human coughing and does not appear to have any harmful effect on the lungs or circulation.

Besides patients with respiratory muscle weakness, artificial coughing is likely to be of greatest value in conditions in which there is prolonged unconsciousness.

RETENTION or aspiration of secretions leads to lung collapse, to the development and persistence of lung infection, and eventually to fatal respiratory insufficiency. The removal of secretions from the lung is of dominating importance in the successful management of prolonged artificial respiration (Kelleher *et alii*, 1956; Blossom and Affeldt, 1956). In the absence of a natural cough, attempts have been made to provide a cough artificially (Barach *et alii*, 1952*a*; Bickerman *et alii*, 1952). Exsufflation, or the sudden release of pressure in the tank respirator after fully expanding the lungs, was found to be moderately effective in removing secretions from the lungs of paralysed patients (Barach *et alii*, 1952*b*; Cherniak *et alii*, 1954). More recently Forbes (1958) has described the "cough bellows", which provide a very convenient form of exsufflation for the respirator patient.

Positive pressure inflation followed by the sudden application of negative pressure via a face mask—exsufflation with negative pressure (E.W.N.P.)—was a more widely applicable and clinically more effective method of artificial coughing (Barach *et alii*, 1953, 1954; Segal *et alii*, 1954; Beck *et alii*, 1955; Williams and Holaday, 1955). Outside the U.S.A. mechanical coughing appears to have received little attention.

The present report describes a simple machine for artificial coughing by E.W.N.P. and its value in the control of lung infection and collapse in patients with respiratory paralysis. Some further observations have been made on lung mechanics during the use of this machine on normal subjects, which will be considered in relation to the safety of the technique.

COUGH MACHINE

The original machine was developed in 24 hours to handle the emergency problems created by respiratory paralysis during the epidemic of poliomyelitis in Western Australia in 1956. It consisted of a high-speed suction-blower, the pressures from which were applied to the patient via a tube and face mask. By means of a hand-operated slide valve, the airway could be rapidly connected to the negative-pressure or suction side of the motor after positive-pressure inflation. Though most of the work to be reported here was performed with this machine, several other models were tested including automatic cycling from positive to negative pressure. Automatic cycling did not appear to confer any advantage, and was abandoned in favour of a simple hand change. The final machine was designed with the help of a local manufacturer[1] for supply to patients with permanent respiratory muscle weakness, and is shown in Figures IA and IB. An extension to one end of a blower motor contains the valve

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[2] Joint Coal Board Research Fellow.
[3] Based on work performed whilst Deputy Medical Superintendent of the Royal Perth Hospital Annexe, Shenton Park, Western Australia.

[1] Avion Products, rear 9 Milligan Street, Perth, Western Australia.

mechanism, which may easily be removed for cleaning. The maximum air-flow rate of 12 litres per second is produced on the negative-pressure or suction phase, but when the machine is turned to positive pressure for inflation, a baffle reduces air-flow rate to approximately 8 litres per second. A very rapid change from positive to negative pressure can be made by hand by means of the lever at the end of the

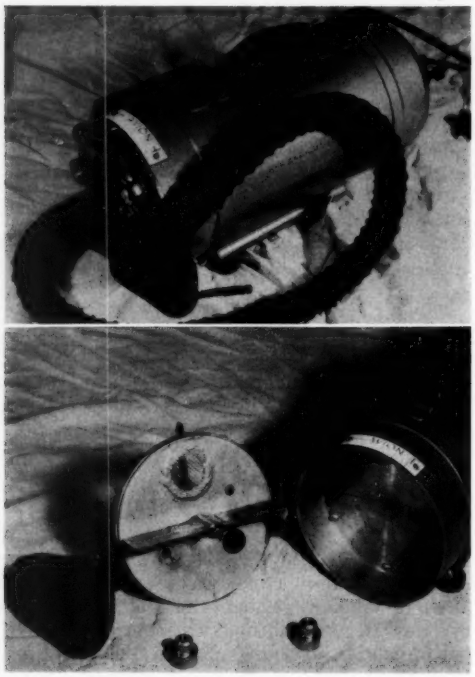


FIGURE I

Above, machine for artificial coughing with attached tube and face mask. Below, the cover removed from the end of the machine, showing simple valve mechanism

machine. The static positive and negative pressures are adjustable and may be checked at any time by connecting the machine to a standard mercury manometer. In the present study the maximum positive pressure used was 40 mm. Hg, with a negative pressure of 40 to 50 mm. Hg.

METHODS

Essentially, artificial coughing with this machine involves inflation of the lungs over 1 to 3 sec. to full expansion at a pressure of 30 to 40 mm. Hg, followed by the sudden application of negative pressure at a high flow rate. The removal of secretions depends on creating a high expiratory flow rate combined with a high expiratory pressure gradient between the alveoli and the mouth. For coughing by face mask, cooperation of the patient is essential, as air flow can easily be stopped in expiration or inspiration by closing the larynx.

The patient was advised to keep his mouth open and to permit air to flow in and out of the lungs passively. The machine could be used, if necessary, by one person, the mask being held firmly on the face with one hand, whilst the pressure change was made with the other. The movements of the chest and abdominal wall were observed as an indication of the degree of inflation. At the moment of full inflation, the pressure was suddenly changed by operating the valve mechanism. The expiration or " cough " phase was complete in one or two seconds when the mask was removed from the face.

Secretions usually collected in the mouth, and were cleared by suction. When large quantities of secretions were produced, they tended to accumulate in the face mask and tubing, which therefore required cleaning after each cough session. To reduce the danger of cross infection, a separate tube and face mask was used for each patient. If a single cough was ineffective, a series of four to six were given with an interval of one minute or so between the series. The total time for one cough session varied from 5 to 30 minutes, according to the quantity of secretion and the rate of removal. Usually three or four sessions each day were sufficient for adequate control of secretions and the clearing of lung collapse. Chest percussion and the head-down position were used with advantage during some coughing sessions.

Ineffective coughing was usually due to the patient's stopping the flow of air at the larynx, or to the operator's not permitting full inflation before application of negative pressure. With experience and explanation, these problems were invariably overcome. On a few occasions air did pass into the stomach ; this was usually due to the inflation phase being continued for too long, and could be avoided by a proper technique. However, even with care, the cough therapy of patients who were in poor condition did occasionally lead to gastric distension, the air being removed by means of a gastric tube.

The machine was also applied via a tracheotomy tube, the technique being very similar to face-mask coughing, with the exception that less patient cooperation was required, as hold-up at the larynx could not occur. No difficulty was experienced in maintaining the

ventilation of patients with complete respiratory paralysis during cough sessions, as with the large inflation produced by the machine only three to six breaths per minute were required for adequate respiratory exchange.

Chest X-Ray Examination of Paralysed Patients

In order to obtain adequate chest X-ray films of patients with respiratory paralysis, their lungs were fully inflated with the Royal Melbourne resuscitator[1] whilst the exposure was made. For this purpose artificial respiration with the tank respirator was temporarily interrupted. Chest films were obtained each day, or more or less often as required.

Clinical Material

All the patients with which this report is concerned were treated at the Shenton Park Annexe of the Royal Perth Hospital during 1956–1957, and had some degree of respiratory

TABLE I

Artificial Coughing by E.W.N.P. for Paralysed Patients with Retained Pulmonary Secretions

Diagnosis	Number of Patients	Artificial Respiration[1]	Major Collapse[2]	Relieved
Poliomyelitis	13	12	7	12
Polyneuritis	3	3	2	3
Cervical spinal cord lesion..	2	—	—	2
Motor neuron disease	1	1	1	1
Tetanus	2	2	1	2
Total ..	21	18	11	20

[1] Tank respirator in 16, rocking bed and/or cuirass in two.
[2] Collapse of at least one lobe as shown by X-ray examination.

paralysis (Table I). This was due to poliomyelitis in 13, 12 of whom required prolonged artificial respiration in the tank respirator. Of these 12, nine had bulbo-spinal paralysis and in seven tracheotomy was performed; three patients had pure spinal paralysis. Two of three patients with acute febrile polyneuritis were maintained on artificial respiration by the tank method; for one a rocking bed was sufficient. Two patients had abdominal and intercostal muscle paralysis due to cervical cord injury. In one patient respiratory muscle weakness without bulbar involvement was due to motor neuron disease. In two patients bulbo-spinal paralysis was produced with "Tubarine" as part of the management of their severe tetanus; both had tracheotomies and artificial respiration by the tank method.

[1] Commonwealth Industrial Gases Ltd., Sydney, N.S.W.

In two normal subjects from the Department of Medicine, University of Sydney, mouth and œsophageal pressures were studied during artificial coughing produced by the machine shown in Figure I. Œsophageal pressure was measured with an air-filled balloon (Mead *et alii*, 1955) with the subject sitting erect; both pressures were transmitted to Sanborn transducers, and after amplification were recorded on a "Sanborn" oscillograph.

RESULTS

Normal Subjects

During inflation in a normal subject (Figure II), the mouth pressure gradually increased to 36 to 38 mm. Hg, whilst the œsophageal pressure rose to 5 to 10 mm. Hg,

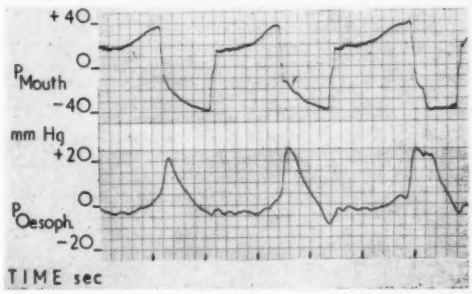


FIGURE II

Simultaneous recording at 25 mm. per second of mouth pressure (P_{Mouth}) and intraœsophageal pressure (P_{Oesoph}), both in millimetres of mercury, during artificial coughing with the machine shown in Figure I

producing a transpulmonary pressure of 26 to 32 mm. Hg. With sudden change to suction, the mouth pressure fell 50 mm. Hg in less than 0·1 sec., the œsophageal pressure showed a further increase followed by a decrease as the mouth pressure became progressively more negative. Similar results were obtained in the other subject tested, except that the further rise in œsophageal pressure did not always occur when the mouth pressure was suddenly reduced. The transpulmonary pressure averaged 27 mm. Hg at a peak mouth pressure of 40 mm Hg.

Patients

In the 1956 poliomyelitis epidemic in Western Australia, 33 patients with respiratory paralysis were treated in the tank respirator. In 12 of these patients coughing by E.W.N.P. was an important factor in the control of lung infection and collapse, and in at least four patients this technique was life-saving. Of five patients in whom lung complications contributed to

death, three did not have artificial coughing, in one the cough bellows was used, and one patient died during coughing by E.W.N.P. (Case V). The results of E.W.N.P. in these and other cases are summarized in Table I. Massive collapse of one lung was relieved on eight occasions in seven patients.

Three patients with respiratory paralysis due to acute polyneuritis required extensive mechanical coughing. In two this treatment was effective in obtaining reexpansion of a collapsed right lung, and in the other (Case VI) frequent coughing over two weeks controlled abundant secretions and collapse did not occur. In two quadriplegic patients, E.W.N.P. was effective on several occasions in controlling lung

TABLE II

Comparison of Coughing by E.W.N.P. with the "Cough Bellows" and Bronchoscopy

	Number of Patients	Relieved	Died
E.W.N.P.	21	20	1
Cough bellows ..	11	1	1 (9 E.W.N.P.)
Bronchoscopy ..	5		1 (4 E.W.N.P.)
No coughing treatment	4	1	3

infection. In one patient with motor neuron disease (Case VII), artificial coughing was essential to survival from an episode of acute respiratory distress precipitated by secretions.

In one patient with post-abortional tetanus, coughing by E.W.N.P. gave complete control of bronchial secretions. No gross lung collapse occurred, and after 11 days of therapeutic paralysis and artificial respiration recovery was uneventful. In one further tetanus patient (Case VIII), prophylactic coughing was inadequate; collapse of the upper lobe of the right lung was subsequently relieved by more vigorous E.W.N.P., but the patient died from renal failure.

In patients with respiratory weakness, the common cold often leads to an increase in bronchial secretions which after a few days become purulent. On 14 occasions in eight patients, lung infection developing in this way was promptly controlled by E.W.N.P. combined at times with penicillin.

Artificial coughing by E.W.N.P. was more effective than by the expiratory-impulse bellows (cough bellows) (Table II). One patient treated with the cough bellows died suddenly. Post-mortem examination revealed interstitial and mediastinal emphysema with pneumothorax due to rupture of a partially collapsed left lung. The peak negative tank pressure in this case was 45 to 50 mm. Hg. Lung complications in nine other patients not relieved with the cough bellows were subsequently relieved by E.W.N.P.

Bronchoscopy with aspiration of secretions on eight occasions in five patients brought only transient benefit. In four of these patients, secretions were controlled and collapse was relieved with E.W.N.P. One patient who did not have artificial coughing died as a direct result of retained secretions and massive collapse. Of three other patients who did not have artificial cough, lung complications contributed to death in two.

REPORTS OF CASES

The following case reports illustrate the place that coughing by E.W.N.P. has in the management of patients with respiratory weakness.

CASE I.—J.B., a female patient, aged 29 years, was admitted to hospital on January 13, 1956, in the acute stage of poliomyelitis. With progression to respiratory paralysis, artificial respiration was commenced two days later in the tank respirator. The next day pharyngeal paralysis with pooling of secretions was observed; secretions were removed by continuous post-nasal suction. By the fourth day respiratory muscle paralysis was complete. At the end of the first week the patient was afebrile, and a chest X-ray film showed clear lung fields. During the second week a gradual increase in tank pressures was required to maintain adequate ventilation and her fever reappeared.

On the eleventh day, severe cyanosis due to respiratory obstruction could not be relieved by suction through the larynx. Coughing by E.W.N.P. was attempted for the first time without result, owing to the patient's inability to cooperate. At bronchoscopy mucopurulent plugs were removed, with relief of her acute respiratory distress. A few hours later, during intermittent positive-pressure respiration for nursing treatment, there was palpable bubbling of secretions in the trachea and major bronchi and very little movement of the left side of the chest. The next day a small amount of mucopurulent secretion was removed during mechanical coughing, which was followed by gross cyanosis. Secretions obstructing the trachea just below the larynx were removed at bronchoscopy and a satisfactory airway was restored. A chest X-ray film showed collapse of the lower lobe of the left lung. On the fourteenth day severe cyanosis again followed coughing by E.W.N.P., and secretions were cleared with difficulty. The next day a large quantity of secretions were removed during two prolonged cough sessions, with great improvement in her general condition. Coughing by E.W.N.P. was omitted on the sixteenth day.. A severe cyanotic attack occurred, which was relieved after bronchoscopic aspiration; but half an hour later bubbling of secretions in the bronchi was again evident. During coughing by E.W.N.P. much more secretion was removed. For the next week artificial coughing gave good control of secretions, but the left lung remained collapsed.

On the twenth-fourth day coughing was omitted and bronchoscopic aspiration was performed, with relief of cyanosis. A chest X-ray examination showed massive collapse of the left lung. E.W.N.P. was

continued; but when four days later the left lung remained collapsed, bronchoscopic aspiration was repeated; no definite benefit followed. Secretions were gradually controlled with artificial coughing, and X-ray examination at the beginning of the seventh week showed partial reexpansion of the left lung. The patient was independent of respiratory assistance ten weeks after her admission to hospital and had no further respiratory difficulty. The left lung was incompletely expanded at six months, but fully expanded one year after her admission.

This patient was the first in whom the removal of secretions from the lungs with the cough machine made a major contribution to treatment. At the same time she presented more difficulties than any subsequent patient. Progressive accumulation of secretions occurred during the first two weeks, so that the withdrawal of a large quantity of secretions during early coughing sessions caused complete tracheal obstruction. With greater experience, this emergency would have been more readily overcome by continued coughing. Bronchoscopy temporarily relieved gross bronchial and tracheal obstruction on three occasions. However, this procedure contributed little to the overall control of copious bronchial secretions.

CASE II.—R.H., a male patient, aged 39 years, was admitted to hospital on February 3, 1956, with acute poliomyelitis and gross bronchial infection. He was cyanosed and in great respiratory distress with complete abdominal and intercostal muscle paralysis. Though there was audible and palpable bubbling of secretions in the chest, no pharyngeal weakness or accumulation of secretions in the throat was demonstrated. An attempt to use the cough machine immediately failed owing to his acute distress and artificial respiration was commenced in the tank respirator. With improvement after half an hour, a cough session was given and the lungs were cleared of secretions. After this his colour became normal.

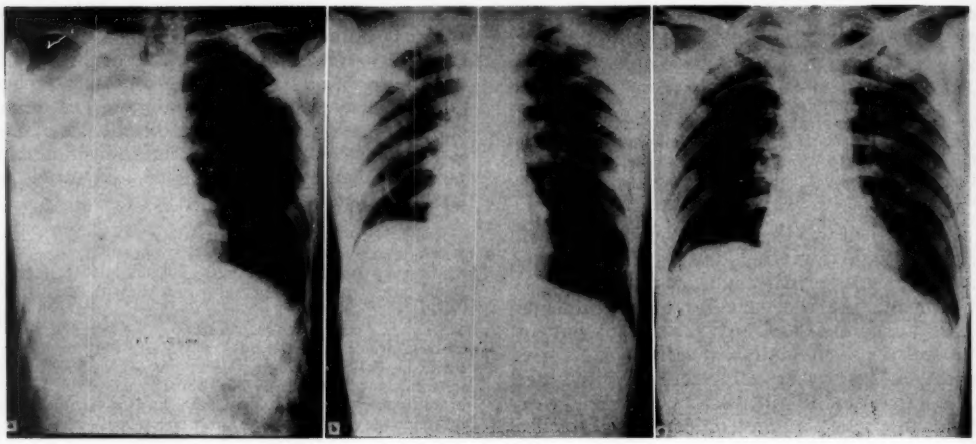
Chest X-ray examination on the third day showed clear lung fields. Coughing by E.W.N.P. during the first eight days was very effective and controlled secretions, but bronchial infection persisted. On several occasions bronchospasm was evident and coughing was more effective after the intravenous administration of 0·24 gramme of aminophylline. Less frequent coughing on the ninth and tenth days was followed by massive collapse of the right lung on the twelfth day. Bronchoscopic aspiration was performed without definite improvement, and massive collapse persisted. More vigorous coughing by E.W.N.P. combined with chest percussion allowed removal of several ounces of purulent secretions. As his infection had not responded to penicillin combined with streptomycin, the exhibition of chlortetracycline was commenced. *Staphylococcus aureus*, hæmolytic and coagulase-positive, was cultured from the secretions removed with coughing. In two days there was improvement in aeration of the right lung, demonstrated radiologically. Nine days after the collapse of the right lung the infection was well controlled, and subsequent X-ray films showed clearing and gradual reexpansion of the right lung. He was independent of respiratory aid five and a half weeks after his admission to hospital, and recovery was uneventful.

In the absence of artificial coughing, this patient would probably have died from the combined effects of purulent bronchitis and respiratory paralysis. It is unlikely that tracheotomy alone would have allowed adequate control of secretions, and with effective coughing it was unnecessary. In this case infection was not controlled by coughing alone, and antibiotic treatment was equally important.

CASE III.—P.T., a male patient, aged 24 years, was admitted to hospital on February 18, 1956, in respiratory failure due to acute poliomyelitis, and was immediately given artificial respiration in the tank respirator. Two days after his admission he suddenly developed complete respiratory obstruction; his trachea was intubated and a tracheotomy was performed. A chest X-ray examination at this time showed clear lung fields. No difficulty was experienced in controlling tracheal secretions, and no serious lung infection developed. One month after his admission, when his vital capacity was less than 100 ml. and he was able to breathe for only one or two minutes unaided, the tracheotomy tube was removed. Eleven days later, after two days of right-sided chest pain, he had massive collapse of the right lung (Figure IIIA). When he was postured head down, coughing by E.W.N.P. was effective in removing a large quantity of purulent secretion. With repeated artificial coughing he was afebrile in 48 hours, when a culture from the sputum had grown *Pseudomonas pyocyanea*, *S. aureus* and *Proteus mirabilis*. Two or three cough sessions were given each day, and after one week there was good aeration of the right lung (Figure IIIB). After three weeks the right lung was completely reexpanded (Figure IIIC).

Because coughing by E.W.N.P. did not immediately cause the clearing of secretions, it was regarded as ineffective. However, with explanation and posturing, success was achieved. The ability to control lung infection and collapse by face-mask coughing allows the removal of the tracheotomy tube from all respirator patients, no matter how small their vital capacity, as soon as the upper airway has recovered normal function.

CASE IV.—A.H., a male patient, aged 35 years, was admitted to hospital on March 20, 1956, suffering from acute poliomyelitis with quadriparesis and respiratory paralysis. Tank respiration was commenced when his vital capacity was 1·1 litres (expected capacity, 4·5 litres). The next day bulbar paralysis was evident and tracheotomy was performed. After a difficult acute stage, which included coma and hypertension (in spite of adequate ventilation), ileus and pulmonary infection, he appeared medically stable one month after his admission. The tracheotomy tube was reduced in size and removed at the end of the fifth week. At this stage his vital capacity was less than 200 ml. and he was able to breathe unaided for two minutes. Fever returned after two days, and four days after removal of the tracheotomy tube he suddenly developed cyanosis and severe respiratory distress with palpable râles over both sides of the chest. Coughing by E.W.N.P. was effective in the removal of purulent secretions. There was slight improvement in colour, followed by rapid deterioration. Further coughing brought only transient improvement, and



(*a*) (*b*) (*c*)

FIGURE III

Case III; (*a*) antero-posterior X-ray film of chest showing massive collapse of the right lung; (*b*) eight days after (*a*), showing clearing of the right lung, but incomplete expansion; (*c*) three weeks after (*a*), showing both lungs expanded and clear

bronchoscopic aspiration was performed. As adequate ventilation had not been restored by these procedures, a tracheotomy tube was reinserted. With the aspiration of more secretions, adequate ventilation was restored. Oxytetracycline therapy was commenced, and he was afebrile in two days.

The day after this attack of obstructive hypoxia, the cough machine was used via the tracheotomy tube for the first time. When a few days later there was further difficulty in maintaining ventilation, an adequate airway was restored by coughing via the tube. Pulmonary secretions were soon controlled, and three weeks after the tracheotomy tube had been reinserted it was again reduced in size and the patient was adapted to face-mask coughing. By this time the X-ray films of his chest which had shown partial collapse of the upper lobe of the left lung, was clear, and in another week the tracheotomy tube was removed. His vital capacity had risen to 300 ml., and he was able to breathe unaided for 15 minutes. Two days later there was complete collapse of the left lung (Figure IVA). After vigorous coughing, a film taken half an hour later on the same day (Figure IVB) showed considerable clearing of the left lung. On this occasion no antibiotics were given, and he was febrile for only 36 hours. With continued coughing, the lungs were clear in two weeks (Figure IVc).

Steady improvement occurred during the next few months, though he continued to require coughing to help remove secretions nearly every day. Increases in bronchial secretions associated with coryza on three occasions were easily controlled. A year after his paralysis, his vital capacity was 800 ml. and he required a cuirass respirator for sleeping. During a further four years, though there was little change in his respiratory power, bronchial secretions continued to be easily controlled with occasional artificial coughing.

If prophylactic coughing had been applied from the time when the tracheotomy tube was first removed, a serious situation would probably not have developed. The inability to relieve obstructive anoxia by E.W.N.P. during the first episode can be related to the inexperience of the operator and the patient. On the second occasion, greater confidence made artificial coughing completely effective.

CASE V.—A male patient, aged 28 years, was treated by the tank respirator combined with tracheotomy for bulbo-spinal paralysis due to poliomyelitis. With recovery of the ability to swallow, the tracheotomy tube was reduced in size and he was adapted to face-mask coughing. The lungs were clear clinically and radiologically when the tracheotomy tube was removed during the eleventh week. Two days later, an X-ray film of his chest showed collapse-consolidation of the lower lobe of the right lung. Treatment with E.W.N.P. and oxytetracycline brought improvement in a week, but did not prevent relapse a few days later. It appeared that face-mask coughing was not fully effective, and as the patient was having manipulation of his left arm whilst anæsthetized, it was decided to make him cough via an endotracheal tube at the same time. The patient was anæsthetized with "Pentothal" and his trachea was intubated with the aid of "Scoline". The first time endotracheal coughing was performed, a large amount of purulent secretion was removed and there were no untoward effects. On the second occasion, two days later, just after coughing had commenced, pallor and absence of arterial pulsation were noted; resuscitation was unsuccessful. Post-mortem examination did not reveal any lung damage due to coughing, and provided no evidence as to the cause of the cardiac arrest.

In the light of reviews of the ætiological factors in cardiac arrest by Stephenson *et alii* (1953) and Ruth *et alii* (1957), the most likely cause in this patient was a vagal reflex, the more so as atropine had not been given. The major

factor provoking this reflex was probably intubation under anæsthesia (Raffan, 1954), but distension of the lungs no doubt increased afferent vagal impulses and may have contributed to the risk of vagal inhibition. Though cardiac arrest in these circumstances would be expected to be an uncommon event, it is doubtful whether, with the small risk, coughing via an endotracheal tube under anæsthesia is justifiable. Where face-mask coughing is ineffective, it would be preferable to perform or restore a tracheotomy and cough by this tube until lung infection is under control.

CASE VI.—A.R., a male patient, aged 51 years, was admitted to hospital on June 3, 1957, suffering from acute polyneuritis. He had respiratory muscle weakness and was unable to clear his lungs of accumulated secretions. For thirty years he had been addicted to heavy cigarette smoking, and more recently had noticed chronic cough with sputum. Artificial coughing was required immediately, and thick purulent secretions were removed, with relief of his respiratory distress. After coughing, his vital capacity was 1·6 litres (predicted 4·6 litres), and artificial respiration was not required.

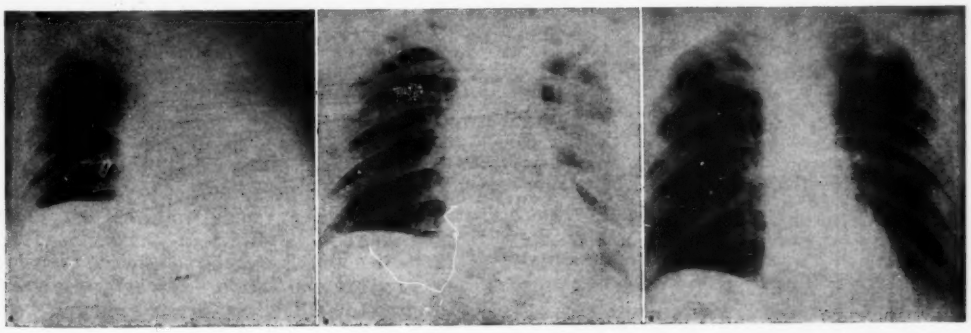
Initially, there was great difficulty in controlling bronchial secretions, for which he required mechanical coughing each 10 to 15 minutes. Aminophylline appeared to make coughing more effective. The vital capacity remained at about 1·6 litres during the first two weeks, variations during this time being related to the accumulation of secretions. A course of penicillin and streptomycin did not have any appreciable effect, and was discontinued after one week. On the morning of the ninth day he suddenly became cyanosed. Frequent coughing by E.W.N.P. was required all day and for prolonged periods. Because of the difficulty with breathing provoked by secretions, he was given continuous respiratory assistance on a rocking bed for five days. Repeated sputum cultures showed a normal bacterial flora. X-ray films of the chest on the second and twelfth days showed clear lung fields. After the first two weeks there was little difficulty in controlling bronchial secretions, and three weeks after his admission his vital capacity had increased to 2·5 litres. Further recovery was uneventful.

Coughing by E.W.N.P. was the most important aspect of the management of this patient, and was effective in preventing any detectable lung collapse. Without an effective means of coughing he would have required tracheotomy and artificial respiration, and even then his survival would have been in doubt.

CASE VII.—W.K., a male patient, aged 35 years, was admitted to hospital on March 26, 1957, in acute respiratory failure. For twelve months he had suffered increasing muscle weakness and wasting accompanied by fibrillation characteristic of motor neuron disease. For the previous two months, after a cold, he had had a productive cough. On the morning of his admission he became suddenly short of breath whilst coughing, and when admitted he was dyspnœic and slightly cyanosed. Chest X-ray examination showed massive collapse of the left lung. Bronchoscopic aspiration relieved his respiratory distress for about half an hour. After further deterioration, the cough machine was used with removal of purulent secretions. Artificial coughing was continued, and on the eighth day a chest X-ray examination showed the left lung to be reexpanded and clear. His vital capacity was 800 ml.; there was complete paralysis of intercostal and abdominal muscles, and a double-exposure chest film showed only about 1 cm. of diaphragm movement. Accessory muscles were continuously in use and probably made the main contribution to ventilation. A double-exposure film showed the greatly increased diaphragm excursion produced during use of the cough machine (Figure V).

Six months later, though his vital capacity had declined to 200 ml., his lung fields remained clear. Respiration was maintained by the use of a rocking bed for 18 hours and frog breathing for six hours each day. Bronchial secretions were easily controlled by occasional use of the cough machine.

This patient with respiratory weakness showed the usual pattern of lung infection and collapse



(*a*) (*b*) (*c*)

FIGURE IV

Case IV; (*a*) antero-posterior X-ray film of chest, showing massive collapse of the left lung; (*b*) half an hour after (*a*), showing re-aeration of the left lung; (*c*) two weeks after (*a*), showing clear lung fields

following a cold. In the absence of an effective means of artificial coughing, his respiratory insufficiency would have quickly led to death.

CASE VIII.—M.F., a man, aged 60 years, was admitted to hospital on March 1, 1957. He had suffered from gout for 17 years and, in the last three days, from the onset of symptoms of tetanus. Chest movement was restricted owing to increased muscle tone, the vital capacity being 1·1 litre (predicted 4·5 litres). Secretions were audible in the trachea and main bronchi. Because of a poor airway and inability to dispose of secretions, tracheotomy was performed.

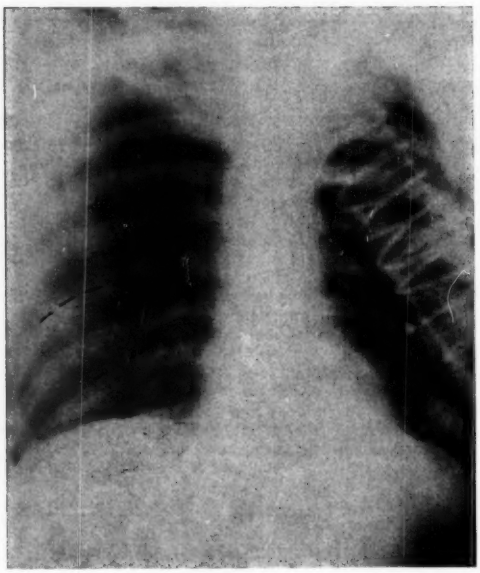


FIGURE V

Case VII; antero-posterior double-exposure X-ray film of chest, showing diaphragm excursion produced during E.W.N.P. from seventh to tenth rib posteriorly on the right, from eighth to tenth rib posteriorly on the left. The interrupted lines show the position of the diaphragm before inflation

As muscle spasms were uncontrolled with chlorpromazine and "Myanesin" and breathing became more difficult, artificial respiration was commenced in the tank respirator after muscular paralysis with "Tubarine". Thick secretions were aspirated from the tracheotomy tube, and a clear airway was maintained with the use of the cough bellows and E.W.N.P. Chest X-ray films on the third, fourth and sixth days showed clear lung fields. On the seventh day more difficulty was experienced in maintaining an adequate tidal volume, and tank pressures were increased. For the next three days secretions were cleared by frequent exsufflation with the cough bellows, E.W.N.P. being used only once each day. On the tenth day there was a gradual fall in blood pressure to 60 mm. Hg systolic, with a general deterioration. The circulatory state was restored with vasoconstrictors and transfusion of blood and serum. X-ray examination on the eleventh day showed collapse of the upper lobe of the right lung. Energetic coughing via the tracheotomy tube by E.W.N.P. was performed three times a day. An X-ray examination two days later showed the upper lobe of the right lung to be expanded. The patient died from uræmia on the sixteenth day. At the post-mortem examination, the bronchial tree was clear of secretions and there was no gross evidence of infection or collapse of the lungs.

Throughout the first ten days of this patient's treatment, E.W.N.P. was used for coughing only occasionally, main reliance for removal of secretions being placed on the cough bellows. This régime was effective at least for the first six days, but did not prevent later lung collapse. The reexpansion of the upper lobe of the right lung within two days after six cough sessions with E.W.N.P. indicates the much greater efficacy of this method.

DISCUSSION

The mechanism of the severe effects of aspirated secretions has recently been discussed (Halmagyi and Colebatch, 1961). In the particular situation of patients with respiratory weakness, and especially when prolonged artificial respiration is required, a method to clear away retained secretions is essential in order to overcome airway obstruction, lung infection and collapse. These complications, previously a major cause of morbidity and mortality among respirator patients, can now be either avoided by prophylactic coughing with E.W.N.P. or else promptly controlled when they develop.

E.W.N.P. was found to be more effective than the expiratory impulse bellows of Forbes (1958). This is only to be expected, as in exsufflation with this bellows the expiratory flow rate and pressure gradient depend on the passive recoil of the distended chest wall and lungs. It may be that control of lung complications would have been obtained in more patients with the use of this bellows alone; however, the risk of further deterioration appeared unjustifiable when E.W.N.P. was available. A further advantage of E.W.N.P. is that the tank respirator is not required, and coughing with this machine can be combined with vigorous chest percussion in difficult cases.

In patients with a tracheotomy, the application of E.W.N.P. via the tracheotomy tube (by means of an extension to the inner tube and standard anæsthetic connexions) was found to give better results than application by face mask. This is in contrast with the results of Beck *et alii* (1955), who recorded a higher flow rate by face mask in patients with a tracheotomy. These findings are probably related to the size of the tracheotomy tube. In the case of the patients considered here, the largest possible tube was

inserted, and when later the tube was reduced in size preparatory to removal, coughing was applied by face mask with the tube blocked. When the tracheotomy tube is used, coughing by E.W.N.P. cannot be frustrated by obstruction at the larynx.

The provision of an effective means of coughing allows removal of a tracheotomy tube as soon as secretions are under control and the patient has clear lung fields, no matter how small the vital capacity. Russell (1956) found difficulty in removing tracheotomy tubes from patients with a vital capacity less than 1 litre because of the accumulation of bronchial secretions. Kelleher *et alii* (1956) have also commented on the difficulty of weaning paralysed patients from their tracheotomy. Although serious lung infection and massive collapse followed removal of the tracheotomy tube in two cases here reported (Cases III and IV), no untoward effects were observed in two other cases, in both of which the vital capacity was less than 100 ml. when the tube was removed. Particular care was taken with prophylactic coughing in these last-mentioned patients.

Early removal of the tracheotomy tube is an important factor in reducing the risk of lung infection—ever present whilst the tube is *in situ* (Trueta and Agerholm, 1956). However, in patients with respiratory paralysis unable to swallow, it has not been found possible to avoid tracheotomy by the use of this cough machine, nor is this result to be expected. Satisfactory control of artificial respiration depends on an adequate and reliable airway which, for these patients, only tracheotomy can provide. Nevertheless, the duration of the tracheotomy can now be greatly reduced, and if prophylactic coughing is practised, it need be no longer than two to four weeks, depending on the rate of recovery of bulbar function, but independent of the rate of recovery of respiratory muscle power.

Bronchoscopy

As experience with E.W.N.P. increased, bronchoscopy was performed less frequently for the removal of bronchial secretions. At times bronchoscopic aspiration did relieve acute obstructive anoxia; but it is clear from the case reports that bronchoscopy contributed little to the overall control of bronchial secretions, and was not by itself effective in securing expansion of collapsed lung on the eight occasions on which it was tried. This experience is similar to that reported by Camarata *et alii* (1956) and by Kinner-Wilson and Stevenson (1957). Had not artificial coughing been available, death might well have followed bronchoscopy in Cases I, II and VII. The only possible value of bronchoscopy is to relieve obstruction due to secretions in the trachea and main bronchi. This is usually only of transient benefit, and as the relief can be more easily achieved with E.W.N.P., bronchoscopic aspiration should rarely, if ever, be performed in patients undergoing artificial respiration. Kinnier-Wilson and Stevenson have abandoned bronchoscopy in the management of bronchitis in patients with respiratory weakness; however, their régime of postural drainage and assisted coughing was not found to be as effective or reliable as E.W.N.P. in clearing secretions.

Effects on the Circulation

The circulatory effects of E.W.N.P. are an important consideration, as patients for whom coughing is required may have impaired circulatory control from the effects of anoxia, hypercapnia and infection. E.W.N.P. may be regarded as a special form of pressure breathing, the circulatory responses to which have been extensively investigated (Maloney and Handford, 1954; Price *et alii*, 1954; Beecher *et alii*, 1943; Berneus and Carlsten, 1955). During positive-pressure breathing, there is a rise in the central venous pressure proportional to the mean airway pressure; a reduction in venous return and cardiac output may follow in a patient with impaired vasomotor reactivity. With E.W.N.P., the brief duration of the positive-pressure phase, the prompt fall in pressure and the negative-pressure phase combine to reduce the mean airway pressure to a negligible value.

Effect on the Lungs

The peak transpulmonary pressure during E.W.N.P. in two normal young adults was at the upper limit of the normal range (Rahn *et alii*, 1946). The further increase in œsophageal pressure following sudden reduction of mouth pressure can be accounted for by contraction of expiratory muscles which would be expected to occur reflexly with a maximal inflation (Hering-Breuer reflex). No other data on the transpulmonary pressure during E.W.N.P. are available, but the rate of fall in mouth pressure is less than that reported by Beck and Scarrone (1956). This may be due in part to contraction of expiratory muscles, which would tend to raise the mouth pressure and thus oppose the fall produced by the machine.

There is still some difference of opinion as to the maximum static pressure that it is safe to apply to the lungs. From studies of the mechanical properties of the human lungs and

thorax, Rahn *et alii* (1946) suggested that a safe range of pressure for resuscitation was between 30 mm. Hg positive and 20 mm. Hg negative. Schaeffer *et alii* (1958) found that lung rupture and air embolism regularly occurred when the pressure in the alveoli was 60 mm. Hg or more above that on the surface of the lungs. The situation in which rupture is most likely to occur is when only part of the lungs is expanding, so that a smaller fraction of the total applied pressure is expended in moving the chest wall and diaphragm and a greater amount is available to expand the lungs. The presence of a collapsed lobe no doubt contributed to the lung rupture reported here following exsufflation in the tank respirator. This experience indicates that inflating pressures greater than 40 mm. Hg should not be used. During normal coughing the pressures developed often exceed 100 mm. Hg (Sharpey-Schafer, 1953; Ross *et alii*, 1955); however, the pressure difference between the alveoli and intrapleural space is not increased during normal coughing, and consequently lung tissue is not stressed.

The situation is a little different during coughing by E.W.N.P. During inflation, pressure applied at the mouth is expended in producing air flow and in overcoming the elastic resistance of the lung and chest wall. The transpulmonary pressure will be only that required to overcome the elastic resistance of the lung, and will therefore be no more dangerous than a normal deep inspiration. The static pressure of 40 mm. Hg cannot be reached until the lungs are fully expanded; at this moment the pressure is abruptly reduced so that the maximum distending force will be applied only for a fraction of a second. At full inflation with the respiratory muscles paralysed or relaxed, the transpulmonary pressure will be the pressure at the mouth minus the pressure required to overcome the elastic resistance of the chest wall and diaphragm. If an active inspiratory effort is made at the same time as the machine is inflating the lungs, a greater distending force will be applied to the alveoli, the normal limit to expansion may be exceeded and lung tissue may be dangerously stressed. On the other hand, contraction of expiratory muscles, which appears to be a more common reaction, besides limiting expansion will reduce the transpulmonary pressure and prevent a dangerous force being applied to the lungs. A further safeguard applies equally whether the respiratory muscles are intact or paralysed; the mask does not form a perfect seal on the face, so that at or before full inflation air escapes around the mask and the maximum static pressure is difficult to achieve.

During expiration, the application of a negative pressure of 40 to 50 mm. Hg is unlikely to have any deleterious effects. The limitation of expiration does not appear to be a decline in transpulmonary expiratory pressure, as a positive pressure may be measured on the surface of the lungs at the end of a forced expiration (Campbell, 1958). Campbell has suggested that expiration is limited by airway closure, which is a consequence of the decline in lung tension (during deflation of the lungs; any increase in intrathoracic pressure can then only maintain the closure. Negative pressure applied to the airway is analogous to positive pressure on the surface of the lungs. It is, therefore, unlikely that more air can be withdrawn from the lungs during the negative pressure phase of E.W.N.P. than after a forced expiration, nor is there any reason to think that this negative pressure is any more damaging to the lungs than the high expiratory pressure gradient which is produced during normal coughing.

Although on theoretical grounds it cannot be established that inflation to 40 mm. Hg during E.W.N.P. in adults will always be safe, lung damage is unlikely. This is borne out by experience, as, when the type of cough machine described here has been used by a large number of different operators, no harmful effects on the lungs have been observed in more than 2000 treatments.

Relation of E.W.N.P. to a Normal Cough

A high expiratory flow rate and tracheobronchial narrowing caused by a high expiratory pressure gradient are the essential features of normal coughing (Ross *et alii*, 1955). The airway narrowing greatly increases the linear velocity of air flow and ensures a forceful expiratory blast. During E.W.N.P., the sudden fall in pressure creates an expiratory pressure gradient similar to that in normal coughing, and at the same time air is withdrawn at a high volume flow rate. The artificial cough differs, in that a high expiratory flow rate is maintained, whereas in normal coughing air is expelled in short bursts. In some patients there is intermittent closure of the larynx during the suction phase of E.W.N.P., and the process then more closely simulates normal coughing.

Forced deflation of the lung has recently been shown to cause a reduction in lung compliance probably due to airway closure (Mead and Collier, 1959). To ensure reinflation of the lungs after E.W.N.P. in patients unable to take a deep breath, a full inflation should be performed with the machine at the end of a cough session.

Although the main application for artificial coughing has been in patients with respiratory muscle weakness, it may be of value for other patients with retained secretions. In prolonged unconsciousness, lung complications are a major contribution to mortality (Maciver *et alii*, 1958). The provision of artificial coughing in these patients would be expected to have the same beneficial effect as in patients with respiratory paralysis. With E.W.N.P. in post-operative patients, Williams and Holaday (1955) found clinical evidence of aeration of areas of massive collapse in a few hours. A further advantage in these patients is the smaller intraabdominal pressures produced during E.W.N.P. compared with normal coughing (Beck and Scarrone, 1956). In congestive heart failure in the elderly, bronchopneumonia developing on the basis of retained secretions is the chief cause of death (Bedford and Caird, 1956). Artificial coughing by E.W.N.P. would solve the difficult problem of providing adequate drainage of secretions and could help to control lung infection in these patients.

Patients with asthma and emphysema may have an impaired cough; however, the limitation of air flow is due to the altered balance of forces in the airways (Dayman, 1951; Campbell *et alii*, 1957; Fry and Hyatt, 1960) and will still be present during E.W.N.P. It is doubtful whether artificial coughing has much to offer for patients fully conscious and with normal respiratory muscle power.

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ADDENDUM

Recently the Avion Cough Machine has been redesigned, and now it incorporates a sensitive gauge which can be switched in to check the applied pressure. The noise level has been reduced, and it is intended to reduce further the air flow rate available during the inflation phase.

STRONTIUM "RICKETS": BONE, CALCIUM AND STRONTIUM CHANGES[1]

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SUMMARY

When rats were fed increasing levels of strontium in the diet, inhibition of calcification was shown by increased width of epiphyseal cartilage, presence of uncalcified bone matrix and decreased ash weight of bone. When ash weight was used as an indicator of mineralization, inhibition of calcification became greater as the dietary strontium level increased. This inhibition became most obvious when the calcium-strontium ratio in serum, expressed on a molar basis, became less than 10 : 1.

In contrast to ash weight, the percentage ash in bone was a less sensitive indicator of rhachitic change, because both mineral and organic weight decreased proportionately, and consequently the percentage ash remained unchanged until high dietary levels of strontium were reached.

The incorporation of strontium into bone varied, not with dietary strontium levels, but with the ratio of calcium to strontium. The two ions were treated similarly, for the decrease in calcium-strontium ratio in the bone was almost directly proportional to that in diet for both young and old rats. In growing animals, addition of new bone crystals was probably the most important mechanism whereby strontium became incorporated into ash, while in slowly-growing rats ion exchange assumed a more important role.

At low dietary levels of strontium, bone matrix formation decreased roughly proportionately to inhibition of calcification, but at higher levels uncalcified bone matrix became prominent. These results may be of significance to the problem of osteoporosis, for they demonstrate that disorder of calcification can be associated with decreased growth of bone matrix. This suggests that "osteomalacic" and "anti-anabolic" mechanisms are not mutually exclusive, but may be interrelated factors in the pathogenesis of osteoporosis.

STRONTIUM has proved a most useful tool in the study of bone; first, as an indicator of the metabolism of bone salts and remodelling of the skeleton (Bauer *et alii*, 1955; Fraser *et alii*, 1960); secondly, in studies of the nature of the calcification mechanism (Sobel, 1954); and finally, as an adjunct in the treatment of osteoporosis in man (Shorr and Carter, 1947; McCaslin and Janes, 1959).

STRONTIUM RICKETS

While small amounts of strontium, because of their chemical resemblance to calcium, can exchange for calcium in the bone crystal (Boyd *et alii*, 1957; Harrison *et alii*, 1957), large amounts inhibit calcification and induce typical rickets in growing rats (Shipley *et alii*, 1922). This form of rickets develops even when adequate calcium, phosphorus and vitamin D are present in the diet (Storey, 1961), although results of different investigators are by no means uniform. Macroscopic signs of rickets may be absent even when the ash content of bones is reduced (MacDonald *et alii*, 1951). Likewise, epiphyseal cartilage width may remain normal, though uncalcified bone matrix has developed in the metaphysis (Follis, 1956). One explanation for these differences is that changes in strontium rickets are not uniformly progressive, but intermittent in nature, with formation of localized osteoid wedges and multiple cartilage nodules in the metaphysis (Storey, 1961).

The nature of the inhibition of calcification by strontium has been investigated extensively *in vitro* by Sobel and his associates, who consider that strontium competes with calcium for a factor in bone whose concentration plays a part in mineralization (Sobel *et alii*, 1935*a*, 1935*b*; Sobel *et alii*, 1949; Sobel, 1954). However, recent tissue-culture experiments do not support such a mechanism (Lengemann, 1957). Thus there is little agreement on the conditions in which strontium inhibits calcification and induces rickets, and further, the mechanism of its rhachitic action has remained obscure. Using strontium-fed rats, the present work is concerned with the relation of dietary and serum strontium and calcium levels to the severity and extent of induced rickets, assessed by histological as well as chemical methods.

[1] Received on May 18, 1961.

[2] In receipt of a grant from the National Health and Medical Research Council of Australia.

EXPERIMENTAL PROCEDURES

Young (40–60 grammes) and adult (200–250 grammes) female rats were divided into groups; one group was fed a basic "Barastoc"[1] diet containing 1·6% calcium and 0·9% phosphorus, the others the same basic diet containing different levels of strontium in the form of strontium carbonate. Strontium carbonate was well mixed into ground "Barastoc" diet, and the mixture was remade into cakes which, together with water, were fed daily to rats. The following dietary levels of strontium were used for both young and adult rats, with the exception that the 1·% level was not used for adult animals: 0·19%, 0·38%, 0·75%, 1·0%, 1·5% and 3·0%. Groups of rats were weighed at the start and end of the experiment.

After 20 days on the diet, animals were anæsthetized with ether, and blood was obtained by jugular incision. This was allowed to clot at room temperature and centrifuged, and the serum was stored at 4 °C. for subsequent analysis. When insufficient serum was obtained for analysis from one animal, serum from different animals in the same group was pooled.

Both tibiæ were dissected from each animal; one tibia was fixed in 10% neutral formol saline, the other stored in acetone. Formalin-fixed tibiæ were decalcified in formic acid, embedded in paraffin wax and sectioned at 7μ; selected bones from both age groups were embedded undecalcified in polyester resin, and sections were cut with a sledge microtome, as described by Ueckert (1960). Sections were stained with Ehrlich's and Weigert's hæmatoxylin, PAS, Alcian blue and Von Kossa's stain.

The width of epiphyseal plates of the proximal ends of the tibiæ were estimated from the histological sections in the following way. The width of each cartilage plate was measured, at a magnification of 100, from the junction of the bone and proliferative chondrocyte zone to the last intact chondrocyte lacunæ in the hypertrophic zone; at least five measurements were made at intervals along the cartilage of each bone. For each dietary level of strontium five separate bones were measured, and the results were expressed as the percentage difference from the mean width of the control epiphyseal plates.

Prior to being ashed, acetone-stored bones were defatted for at least three days in several changes of warm acetone. The ash content of bones was determined by first drying to constant weight at 60° C. and then ashing in platinum crucibles to constant weight in a muffle furnace at 700° C. The weighed ash was then dissolved in warm normal hydrochloric acid preparatory to further analysis.

The levels of calcium and strontium in bone ash and serum were determined with a "Unicam" spectrophotometer (SP900). At the levels of strontium used in the present experiments, it was found that the additive method described by Harrison (1958) for calcium was applicable to strontium estimations directly on diluted serum and ash solutions.

[1] A local proprietary animal food.

RESULTS

The results are shown in Table I for young rats and Table II for adult rats.

Weight Change

As the dietary level of strontium increased, young rats gained less weight. Control adult rats gained less weight than young ones, and those on strontium were less affected than young animals; only at the highest level of strontium used (3·0%) was there an apparent decrease in weight gain.

Width of Epiphyseal Cartilage

In young rats, as the dietary strontium level increased, the epiphyseal plate widened; however, for dietary strontium levels above 0·75%, the cartilage plate became so irregular that

TABLE I

Variations in Weight, Serum Calcium and Strontium Levels and Composition of the Tibia in Young Rats Fed Different Levels of Strontium in a Basic Diet Containing 1·6% Calcium and 0·90% Phosphorus for 20 Days

Strontium in Diet (Percentage)	Weight Change (Percentage Change from Original Weight)	Serum Levels (mg. per 100 ml.)[1]		Bone Composition						
				Dry Weight (mg.)	Ash Weight (mg.)	Ash in Bone (Percentage)	Calcium in Ash		Strontium in Ash	
		Calcium	Strontium				(mg.)	(Percentage)	(mg.)	(Percentage)
0	150 (5)	10·7±0·2[1] (5)		168±7 (8)	86·3±4·0 (8)	51·0±0·6 (8)	31·3±0·7 (8)	36·3±0·5 (8)		
0·19	145 (5)	10·6±0·1 (3)	0·63±0·04 (3)	157±10 (5)	81·2±8·0 (5)	51·8±0·7 (5)	28·6±1·8 (5)	35·2±0·8 (5)	1·2±0·3 (5)	1·4±0·2 (5)
0·38	139 (5)	10·5±0·1 (5)	1·2±0·1 (5)	148±6 (8)	77·3±3·1 (8)	52·2±0·7 (8)	27·1±1·4 (8)	35·1±0·7 (8)	2·4±0·2 (8)	3·1±0·2 (8)
0·75	134 (5)	10·5±0·3 (5)	2·6±0·1 (5)	137±10 (10)	68·6±5·0 (10)	50·0±0·8 (10)	21·8±2·8 (5)	31·8±0·9 (5)	2·8±0·3 (5)	4·1±0·2 (5)
1·0	135 (5)	10·7±0·3 (3)	3·1±0·1 (3)	130±12 (5)	65·8±5·8 (5)	50·7±0·6 (5)	21·4±1·8 (5)	32·5±0·7 (5)	2·8±0·3 (5)	4·3±0·1 (5)
1·5	114 (5)	9·1±0·1 (5)	4·7±0·2 (5)	117±5 (5)	54·2±3·0 (5)	46·3±0·6 (5)	17·6±1·2 (5)	32·4±0·6 (5)	2·8±0·2 (5)	5·2±0·3 (5)
3·0	63 (5)	9·5±0·1 (5)	9·4±0·2 (5)	99±11 (5)	40·2±3·1 (5)	40·7±0·3 (5)	13·0±0·6 (5)	32·3±0·8 (5)	2·0±0·1 (5)	5·9±0·4 (5)

[1] Mean±standard error. The number of animals used in each group is given in parentheses.

TABLE II

Variations in Weight, Serum Calcium and Strontium Levels and Composition of the Tibia in Adult Rats Fed Different Levels of Strontium in a Basic Diet Containing 1·6% Calcium and 0·90% Phosphorus for 20 Days

Strontium in Diet (Percentage)	Weight Change (Percentage Change from Original Weight	Serum Levels (mg. per 100 ml.)[1]		Bone Composition						
		Calcium	Strontium	Dry Weight (mg.)	Ash Weight (mg.)	Ash in Bone (Percentage)	Calcium in Ash (mg.)	Calcium in Ash (Percentage)	Strontium in Ash (mg.)	Strontium in Ash (Percentage)
0	11·1	10·5±0·4[1]		379±33	227±19	59·6±0·3	83·1±8·4	36·6±0·8		
0·19	10·1	10·6±0·3	0·72±0·02	349±19	210±13	60·2±0·3	74·8±2·8	35·6±1·1	1·12±0·06	0·53±0·05
0·38	8·7	10·4±0·3	1·4±0·2	345±9	206±5	59·7±0·8	72·3±2·6	35·1±0·5	1·53±0·09	0·74±0·03
0·75	6·7	10·8±0·1	2·5±0·2	353±35	215±22	60·9±0·2	74·2±7·5	34·6±0·7	1·86±0·18	0·86±0·02
1·5	8·5	9·5±0·1	3·9±0·4	364±32	211±18	57·9±0·4	73·3±5·5	34·7±0·7	2·13±0·21	1·00±0·06
3·0	5·8	8·6±0·2	6·0±0·5	319±8	184±2	57·6±0·9	65·9±8·1	35·7±0·2	2·15±0·06	1·16±0·03

[1] Mean±standard error. Three rats in each group.

measurements were not reliable (Figure I). The epiphyseal plate in control adult rats is much narrower than that in young animals, and showed little change with increasing dietary levels of strontium until the 3·0% level; here the cartilage plate was irregularly widened.

Histological Changes

Young Rats.—The epiphyseal plate in control animals is uniformly wide, and the intercellular matrix is calcified around the last two or three hypertrophic chondrocytes. The metaphysis consists largely of calcified bone trabeculæ, in which osteoid seams are extremely narrow or absent. In the bone shaft, both resorption and lamellar bone formation are seen. Bone resorption is associated with increased vascularity and the presence of osteoclasts and, at the margins of trabeculæ, with absence of uncalcified bone matrix (Figures II and III).

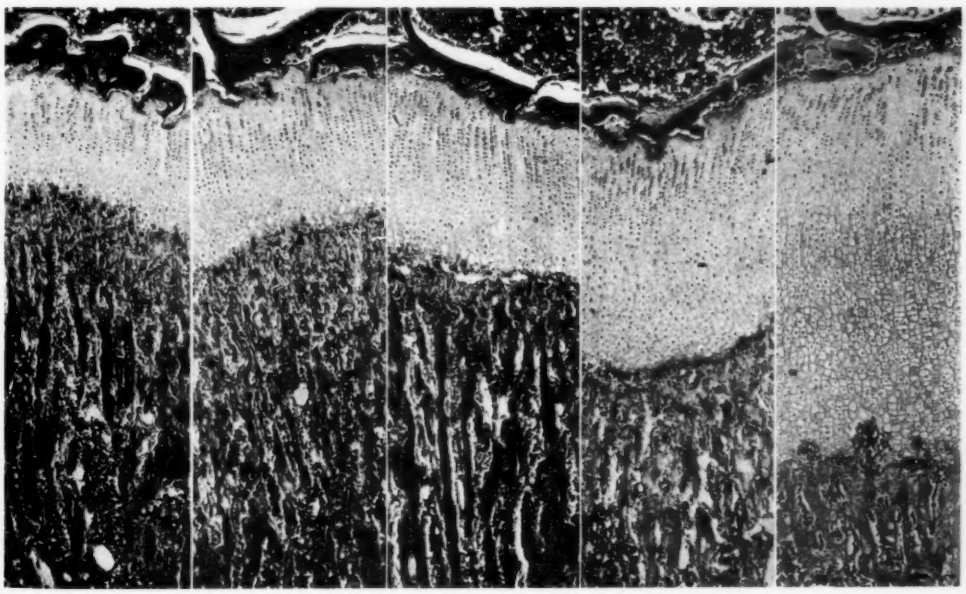

FIGURE I

Photomicrographs showing successive increase in width of the epiphyseal cartilage with increasing dietary levels of strontium fed to young rats for 20 days; from left to right, the dietary strontium levels are as follows: 0%, 0·38%, 0·75%, 1·5% and 3·0%. (Weigert's hæmatoxylin stain. ×25)

In strontium-treated animals no change occurs until the 0·38% dietary level is reached. Here the cartilage plate is irregular and slightly widened, with small areas of uncalcified bone matrix in the lower ends of the metaphyseal trabeculæ and upper end of the diaphysis.

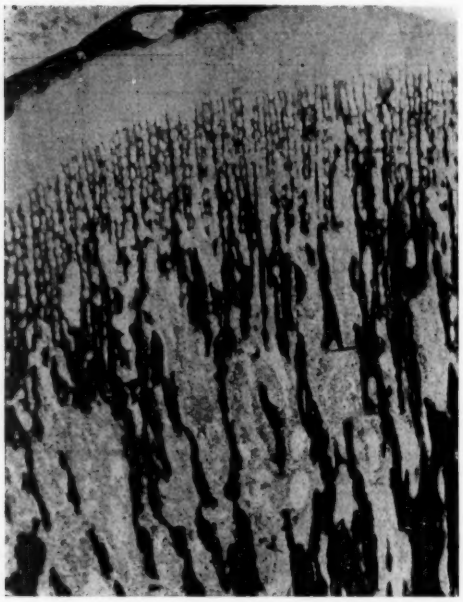

FIGURE II

Photomicrograph of undecalcified section of tibia in a young control rat showing normal endochondral ossification; calcified areas are black. (Von Kossa, Eros stain. ×25)

With 0·75% and 1·0% strontium in the diet, the epiphyseal cartilage is still wider and, in the hypertrophic zone, irregular in outline, with cartilage extensions in the metaphysis. In these projections the zone of calcified intercellular matrix is wider than normal, and at the junction of the metaphysis and cartilage the intercellular matrix columns are no longer parallel, but distorted in arrangement, with occasional groups of flattened chondrocyte lacunæ. Osteoid seams on trabeculæ, particularly in the upper end of the diaphysis, are more prominent than in the previous group of animals.

At the 1·5% dietary strontium level the pattern of endochondral ossification changes. The cartilage is almost double the normal width, and calcification no longer proceeds regularly along the intercellular matrix; instead, small areas of calcification occur in a transverse band across the epiphyseal plate or in the lateral aspects of the cartilage (Figure IV). The greater part of the intercellular matrix of the hypertrophic zone adjoining the metaphysis is uncalcified, and chondrocyte lacunæ are flattened. The pattern of vascular penetration is now different; instead of single vessels growing along columns of cells in the hypertrophic zone, small vascular tufts from the metaphysis grow through the flattened chondrocyte layer and penetrate the cartilage at different sites. Where this occurs, both chondrocytes and intercellular matrix of the cartilage are replaced by uncalcified bone matrix (Figure V). The metaphysis consists of remnants of calcified cartilage cores encased by osteoid tissue, which extend for a considerable distance along the diaphysis. Resorption and remodelling of the bone shaft are conspicuously diminished in extent.

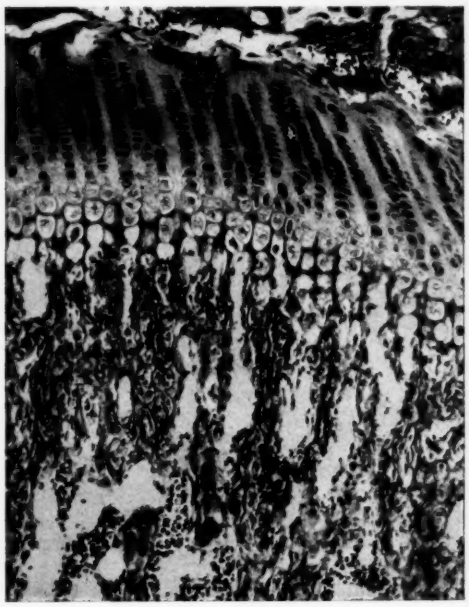

FIGURE III

Photomicrograph of undecalcified section of the tibia from a young control rat showing deeply stained intercellular matrix in the zone of calcification of the cartilage and bone trabeculæ; little osteoid is present at margins of bone trabeculæ. (Ehrlich's hæmatoxylin and eosin stain. ×60)

Similar but more extensive changes are seen with 3·0% strontium in the diet; the epiphyseal cartilage is greatly widened and the hypertrophic zone extremely irregular in outline (Figure VI). Calcification of areas of epiphyseal

cartilage occur, and here localized areas of endochondral ossification are prominent (Figure VII). In some cases long tongue-like processes of uncalcified cartilage extend down into a metaphysis consisting largely of osteoid tissue encasing a few remnants of calcified cartilage and bone. Remodelling and resorption are decreased, not only in the metaphysis, but throughout the entire bone.

Adult Rats.—The first obvious change occurs at the 1·5% dietary strontium level; here the epiphyseal cartilage is slightly wider than normal, and metaphyseal osteoid seams are irregularly increased in extent and width. By the 3·0% strontium level the cartilage plate is appreciably wider, although calcification of the intercellular matrix of the hypertrophic zone continues. The metaphysis consists of a

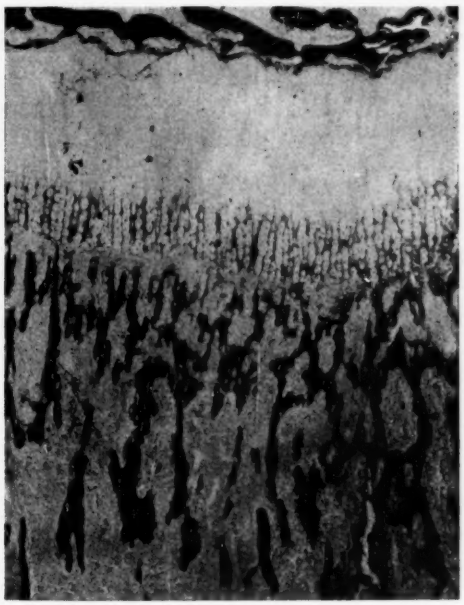

FIGURE IV

Photomicrograph of undecalcified section of the tibia from a young rat fed 1·5% strontium in the diet for 20 days. The epiphyseal cartilage is wider than normal and the zone of calcification irregular in width and outline. (Von Kossa, Eros stain. ×25)

sharply-defined transverse band of osteoid tissue extending down into the upper end of the diaphysis. Throughout the bone, particularly around the majority of vascular canals, are well-defined zones of osteoid tissue, and, concomitantly, areas of bone resorption are decreased in extent and number.

Serum Calcium and Strontium

Young Rats.—As the percentage of strontium in the diet increased, the serum strontium level rose progressively until at a dietary level of 3% strontium it equalled that of the serum calcium.

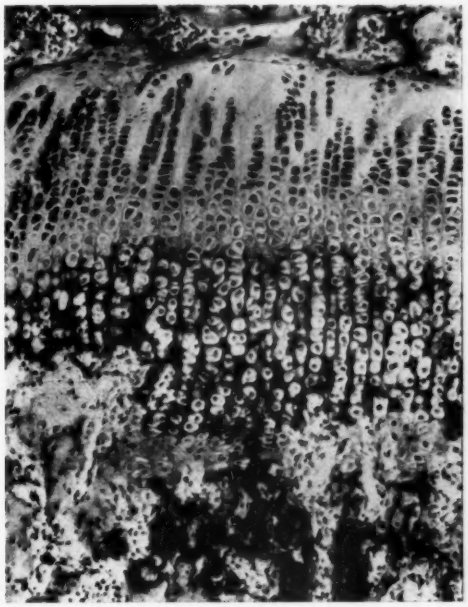

FIGURE V

Photomicrograph of undecalcified section of the metaphysis from a young rat fed 1·5% strontium in the diet for 20 days. A wide zone of calcification in the intercellular matrix of the cartilage plate is in the process of being replaced largely by osteoid tissue. (Ehrlich's hæmatoxylin and eosin stain. ×60)

The serum calcium level showed little change, except for a slight decrease with high dietary strontium levels.

Adult Rats.—As the percentage of strontium in the diet increased, the strontium level in the serum of adult rats rose less rapidly than in young animals. At the maximum dietary level of strontium (3·0%), the serum level was only 6·0 mg. per 100 ml., while the calcium level had fallen to 8·6 mg. per 100 ml.

Bone and Bone Ash Weights

Young Rats.—With increasing levels of strontium in the diet, the tibia gained less weight than in control rats during the twenty-day experimental period. With lower levels of strontium, this was due to a proportional decrease in both ash and organic weight of

bone; only with high levels of strontium (1·5% and 3·0%) did the decrease in ash weight become progressively less than that of organic weight (Figure VIII). This difference was again demonstrated when the amount of ash was calculated as a percentage of the weight

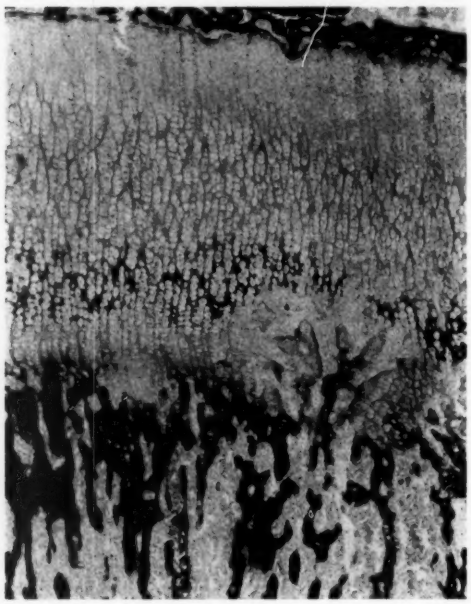

FIGURE VI

Photomicrograph of undecalcified section of the tibia from a young rat fed 3·0% strontium in the diet for 20 days. The epiphyseal cartilage is extremely wide, with a transverse band of calcification in the hypertrophic zone; part of this band has undergone endochondral ossification and is replaced by osteoid tissue. (Von Kossa, Eros stain. ×25)

of dry bone. Here the percentage ash remained within normal limits until the 1·0% dietary strontium level, but, with greater amounts of strontium, it became significantly less. The decrease in ash weight was particularly rapid as the serum calcium-strontium ratio became less than 10 : 1; this is illustrated in Figure IX, which shows the change in organic and ash weights of the tibia in relation to the serum calcium-strontium ratio calculated on a molar basis.

Adult Rats.—The weight and percentage of ash in the control tibia were higher than that in young rats. With strontium administration, there was no consistent pattern of change in ash weight, while the percentage ash in bone showed no appreciable decrease until the 1·5% to 3·0% dietary levels were reached.

Calcium and Strontium in Bone Ash

Young Rats.—With increasing dietary levels of strontium, the amount of strontium in the bone ash rose to become relatively constant from the 0·38% to the 0·75% dietary levels; with further increase in dietary strontium levels, the amount in ash became less. In contrast to this, the strontium level in bone ash, when expressed as the percentage, increased until a constant level was reached at the 1·5% dietary level; this increase was not proportional to, and became less with, each successive increment of strontium in the diet until a value of approximately 5% in ash was reached. This increase in strontium percentage in ash was associated with an almost equal decrease in calcium percentage in ash. The change in calcium-strontium ratio in the bone ash with

FIGURE VII

Photomicrograph of undecalcified section of the tibia from a young rat fed 3·0% strontium in the diet for 20 days. Part of the epiphyseal cartilage has been replaced by osteoid tissue. In the metaphysis deeply-stained cartilage matrix and bone are largely encased by osteoid tissue. (Hæmatoxylin and eosin stain. ×60)

increasing dietary levels of strontium is shown in Figure X, which demonstrates that the calcium-strontium ratio decreased rapidly with low levels and became constant with 1·5% and 3·0% dietary strontium levels. In contrast to this, the decrease in the calcium-strontium

ratio in bone ash was almost proportional to the decrease in calcium-strontium ratio in the diet (Figure XI).

Adult Rats.—The strontium uptake in the tibia of old rats was less than in young rats, and in contrast to young rats, increased progressively with increase in dietary strontium level. The calcium-strontium ratio in bone ash both

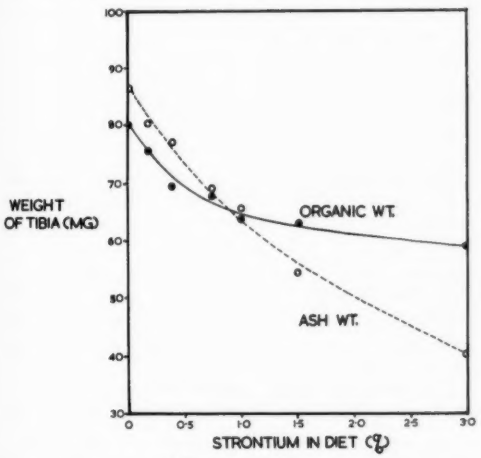

FIGURE VIII

Graph showing the relation between organic and ash weights of the dry defatted tibia and the dietary level of strontium fed to young rats for 20 days

decreased and changed less abruptly than that in young rats, and did not approach a constant value with low calcium-strontium ratios in the diet (Figures IX and X).

Strontium-Calcium Observed Ratio (OR)

Comar *et alii* (1956) introduced the term " observed ratio " (OR) to designate the overall discrimination in metabolic utilization of the elements calcium and strontium from one phase to another in a biological system. The OR is given by the following expression :

$$\text{Observed ratio } \left(\frac{\text{sample}}{\text{precursor}}\right) = \frac{\text{Sr-Ca sample}}{\text{Sr-Ca precursor}}.$$

The OR as defined by Comar *et alii* (1956) is applicable only to situations in which equilibrium has been established between various compartments containing calcium and strontium. The results of Gran (1960), and further experiments with large amounts of stable strontium, demonstrated that the OR for blood to diet was relatively constant after four to eight days (Storey, 1961). Therefore the OR's for blood to diet were calculated in the present experiments. These calculations showed that the OR for blood to diet was of the same order (0·50) for all dietary levels of strontium used in young animals ; by contrast, in adult animals the OR fell progressively as the strontium level in the diet increased.

DISCUSSION

The present work has shown that histological methods are a far more sensitive indicator of the degree of bone calcification than is the change in percentage ash of dried bone. If the percentage ash is used as an index of " rhachitic " bone, no change is detectable below a relatively high critical dietary level of strontium. In contrast, at low dietary levels of strontium, histological examination shows both widened epiphyseal cartilage and, in undecalcified sections, some widening of osteoid seams on trabecular margins. Indeed, if cartilage width is used as an indicator of inhibition of calcification, increasing dietary levels of strontium progressively inhibit mineralization. The explanation for the relative insensitivity of

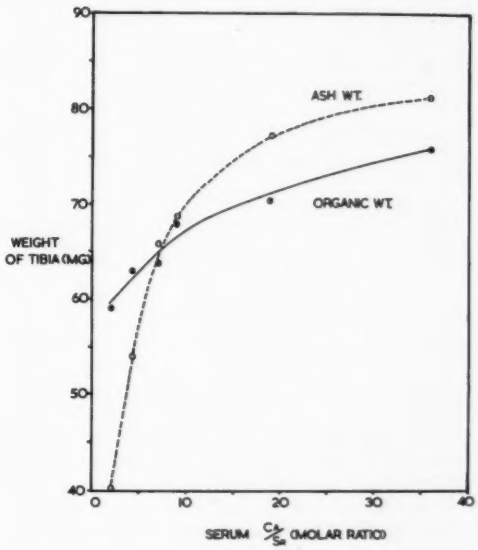

FIGURE IX

Graph showing the relation between organic and ash weights of the dry defatted tibia to the serum calcium and strontium ratio expressed on a molar basis in young rats fed different dietary levels of strontium for 20 days

percentage ash as an indicator is found when organic and inorganic weight changes are compared at the end of the twenty-day experimental period. Analysis of control bones at the beginning of the experiment showed that the ash weight in the normal tibia more than doubled in 20 days. However, in young rats a

progressive decrease in both ash and matrix weight of the tibia occurs with increasing dietary levels of strontium until the 1·0% level is exceeded; thereafter, although bone matrix weight remains relatively constant, ash weight continues to decrease until, with the maximum dietary level of strontium used (3·0%), no increase in ash weight occurs in 20 days. Thus,

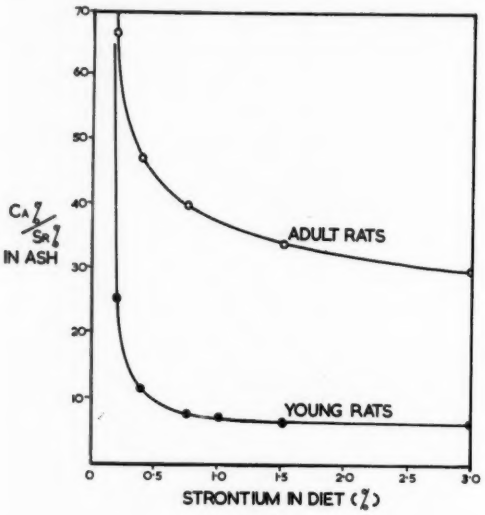

FIGURE X

Graph showing the relation between the percentage weights of calcium and strontium in the ash of the dry defatted tibia and the levels of strontium fed to young and adult rats for 20 days

while a significant inhibition of calcification occurs with low dietary levels of strontium, little discrepancy between growth of matrix and its mineralization is disclosed by a fall in the percentage ash of dried bone. The less extensive changes in adult rat bones are consistent with the finding that wide osteoid seams can form on bone trabeculæ in the metaphysis without alteration in width of the epiphyseal plate (Follis, 1956).

The nature of the changes in the cartilage plate alters as the dietary strontium level increases. At low levels the epiphyseal cartilage is uniformly widened, but with 1·0% of strontium in the diet localized metaphyseal extensions develop, in which chondrocyte capsules are flattened or intercellular columns are irregularly arranged. Here vessels no longer grow along chondrocyte columns as they do in the adjoining plate, so that endochondral ossification is inhibited. That such localized extensions can develop when the blood supply is interrupted has been shown experimentally by Trueta and Amato (1960).

As the strontium level increases still further, flattened chondrocyte lacunæ accumulate to form a layer in the hypertrophic zone, and both orderly ingrowth of vessels and endochondral ossification cease; instead, isolated vascular tufts grow between layers of flattened cells, so that localized centres of ossification occur at the margins and within the epiphyseal plate. This phenomenon has been described in detail by Park (1939) in avitaminosis D rickets, and it appears a necessary prelude to spontaneous calcification of large areas of the widened epiphyseal cartilage. It is likely that this change in structure and pattern of calcification may be due, in part, to mechanical factors operating on a weakened intercellular matrix. That localized changes can occur in the epiphyseal cartilage when the usual chemical evidence of rickets is not present may have important implications. This is particularly pertinent now that it has been shown in long-standing strontium rickets that gross localized epiphyseal defects develop in the form of osteoid metaphyseal wedges and multiple cartilage nodules (Storey, 1961).

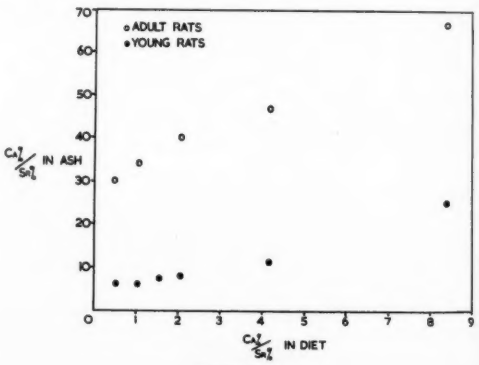

FIGURE XI

Graph showing the relation between the percentage weights of calcium and strontium in ash of the dry defatted tibia and the percentage weights of calcium and strontium in the diet fed to young and adult rats for 20 days

Inhibition of mineralization of embryonic bone occurs with such low strontium levels *in vitro* that Lengemann (1957) concluded that a competitive mechanism could not explain the action of strontium on calcification. In the present experiments, the degree of inhibition of calcification was not of the same order at lower strontium levels as that found *in vitro* by Lengemann (1957). This may be due to inherent differences between in-vitro and in-vivo experiments, to the phenomenon of " delayed " calcification, or to differences in rate of growth between embryonic and young bone. With

high levels of strontium *in vitro*, Sobel *et alii* (1935*a*) found that a molar calcium-strontium ratio of 8 : 1 to 10 : 1 completely inhibited calcification; this ratio is close to that reached in serum in those of the present experiments in which bone ash was significantly affected. It is probable that inhibition at this serum ratio is even more complete than the results show, for strontium does not stop mineralization indefinitely but merely delays it. This "delayed" calcification was clearly demonstrable in the present experiments in widened epiphyseal cartilage, and has been observed *in vitro* by Sobel *et alii* (1935*a*). The results suggest that when the calcium-strontium ratio falls below a critical level, bone crystals cannot form (at least for some time), for there is a limit to the amount of strontium which can become incorporated into hydroxyapatite. This is reached at a calcium-strontium ratio of 9 : 1 (MacDonald *et alii*, 1951; Boyd *et alii*, 1957; Storey, 1961), when X-ray diffraction studies demonstrate gross distortion of crystal lattice pattern (MacDonald *et alii*, 1951). Moreover, with lower calcium-strontium ratios in solution, crystal formation is probably completely inhibited (Engström *et alii*, 1958).

If there is a critical calcium-strontium ratio, below which crystal formation cannot take place, then how does mineralization subsequently occur? That it does so despite calcium-strontium levels which inhibit mineralization in the first instance has been shown *in vitro* by Sobel *et alii* (1935*a*), and *in vivo* in this and other studies (Storey, 1961). Evidence from recent work demonstrates that discrimination against strontium in favour of calcium takes place in bone (Likins *et alii*, 1959; Lengemann, 1957), in contrast to earlier findings that this did not occur (Bauer *et alii*, 1955; Comar *et alii*, 1956). Likins *et alii* (1959) have shown that although strontium uptake by existing crystals favours strontium slightly more than calcium, the rate of release of strontium from crystals is much faster than that of calcium, with overall discrimination in favour of calcium. If such results are applicable to the calcification process, as distinct from exchange in already formed crystals, then this could account for delayed calcification of cartilage and osteoid even when the ratio of calcium to strontium is high enough to inhibit mineralization in the first instance. As to whether this phenomenon is a purely physiochemical process based on differences in ionic radii of calcium and strontium or a more subtle biological process cannot be answered from the present work. However, recent findings of Lengemann (1960) support the hypothesis that vital processes are implicated, for the discrimination process is heat-labile and sensitive to some enzyme inhibitors.

The incorporation of dietary strontium into bone varies, not with dietary strontium levels, but with the ratio of calcium to strontium, strontium being treated similarly to calcium in both young and old rats. However, in young rats fed large amounts of strontium in the diet, the calcium-strontium ratio in bone approaches a constant value. This probably reflects a temporary saturation of the mechanisms by which ions are incorporated into the skeleton, for it takes longer than 20 days for maximum uptake of strontium into bone (Storey, 1961). The length of time required for the calcium-strontium ratio to become constant is probably due to the relatively slow rate at which intracrystalline exchange and recrystallization of imperfectly formed bone crystals proceed *in vivo*.

Two mechanisms, operating at different rates, are thought to account for uptake of strontium into bone; initially, a rapid process of ion exchange of strontium for calcium occurs into the hydration layer of crystals, and, when this becomes saturated, further increase of strontium takes place by slower processes, such as intracrystalline exchange, crystal growth and new bone formation (MacDonald *et alii*, 1951). The present experiments show that the relative importance of the mechanism operating depends on the dose level of strontium in the diet. With high levels, little new ash is added to existing bone in 20 days, and histological study has confirmed that remodelling and resorption of bone, in contrast to cartilage, are largely inhibited; here ion exchange is probably the more important mechanism by which strontium is incorporated into bone. The histological findings eliminate the possibility that new ash formation continues and a constant ash weight is maintained by an increased rate of bone resorption and remodelling. With low dietary levels of strontium, formation and growth of crystals become more important, for the weight of bone ash nearly doubled in the 20 days of the experiment, and thus must account for a considerable proportion of the strontium present in bone. The importance of growth as a means of incorporating strontium into the skeleton is further shown by the smaller amounts present in slowly-growing adult rats; these findings are consistent with those of Weikel and Neuman (1961), who consider that accretion of new mineral is the greatest source of incorporation of dietary calcium into the skeleton of both young and adult rats.

The basic mechanisms of bone growth and calcification studied in these present experiments are significant to the problem of osteoporosis in at least two ways. First, inhibition of

resorption and remodelling of bone is most obvious when dietary strontium levels are high; even in adult rats, most vessels in bone are circumscribed by widened osteoid seams, and bone resorption is almost completely inhibited. As to whether decreased remodelling is primarily due to an increased surface area of bone covered by wide osteoid seams resistant to resorption, or to compensatory inhibition of parathyroid activity associated with the increased level of calcium and strontium ions in blood serum, has still to be determined. If such inhibition of resorption and remodelling occurs when strontium is administered for the treatment of osteoporosis in man, this could provide a rational basis for successful clinical results (Shorr and Carter, 1947; McCaslin and Janes, 1959). Secondly, with low dietary levels of strontium, although calcification is inhibited slightly, growth is decreased also, so that unless special undecalcified sections of bone are used, a small increase in the amount of undecalcified bone matrix is not clearly demonstrable. Thus, if routine histological or chemical procedures are used, the obvious defect may be decreased bone growth until inhibition of mineralization becomes large enough to be detected easily.

This interrelation of calcium metabolism and bone growth deserves further study, in view of recent experiments demonstrating the importance of calcium deficiency in the development of experimental osteoporosis (Harrison and Fraser, 1960). Furthermore, the present experiments suggest that in the presence of abnormal calcium metabolism, whatever its cause, the development of "osteoporosis" or "osteomalacia" depends on the rate of matrix formation.

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THE EFFECTS OF PROLONGED CORTICOSTEROID THERAPY

AN APPRAISAL OF 66 CASES[1]

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SUMMARY

Sixty-six patients suffering from various diseases who received more than 10 grammes of cortisone for a period of more than three months were assessed for response to treatment and incidence of complications.

Long-term corticosteroid therapy in this series was associated with a 60% incidence of complications, and a mortality rate of 6% attributable to corticosteroids. The complications were frequently insidious in onset. The incidence of complications was not influenced by age or sex, but increased with increasing total dose and duration of corticosteroid therapy. A favourable initial response to cortisone was associated with fewer complications. Particular complications were found more frequently in some diseases than in others; dyspepsia and peptic ulcer were most frequently found in rheumatoid arthritis and psychological change in systemic lupus erythematosus. The incidence per patient for complications from corticosteroid therapy was greatest in rheumatoid arthritis and systemic lupus erythematosus, and lowest in chronic hepatitis.

IN this paper an attempt has been made to evaluate the risks of prolonged treatment with corticosteroids by recording the progress and incidence of complications in 66 patients. For the sake of simplicity, when reference is made to dosage an attempt has been made to render doses in terms of cortisone.

CASES STUDIED

Criteria for Inclusion

This survey covers the experience of the Clinical Research Unit of the Royal Melbourne Hospital since the introduction of corticosteroid therapy. With the exclusion of patients who received corticosteroids for brief periods (less than three months), and in low total dosage (less than 10 grammes), there remain 72 cases. Six of these were excluded because of insufficient documentation. Cases of Addison's disease were also excluded.

The final 66 cases included cases of systemic lupus erythematosus (24), rheumatoid arthritis (13), active chronic hepatitis (11), nephrotic syndrome due to glomerulo-nephritis (five), and a miscellaneous group of 13 cases, as follows: sarcoidosis (three), thyroiditis (two), asthma (two), ulcerative colitis (two), hæmolytic anæmia (one), anorexia nervosa (one), cardiomyopathy (one) and polyarthritis (one).

[1] Received on January 11, 1961.

[2] Drug Houses of Australia Fellow.

DURATION OF THERAPY AND DOSAGE

The duration of therapy in these cases varied from three months to seven years (average 30 months), and the total dose of cortisone given varied from 10 to 200 grammes (average 69 grammes). The frequency distribution of duration of treatment and dose is shown in Figure I.

RESULTS

Response to Treatment

The response of each patient was assessed as follows: "good", denoting rapid and sustained improvement; "fair", denoting slow or slight improvement; "poor", denoting no improvement. When chronic diseases are treated with cortisone, there is often an initial good response which may not be fully sustained, the disease being merely alleviated. For this reason, the degree of immediate response was an important factor in determining the group into which a particular case would be placed.

The frequency of each type of response in the various disease groups is shown in Table I. A good response to cortisone was obtained in 22 cases, in which an average daily dose of 66 mg. was given over a period of 32 months; the incidence of complications was low in this group (0·8 per patient), and only two patients died. A fair response to cortisone was obtained in 31 cases, in which an average daily dose of 74 mg. was given for an average period of 31 months; the incidence of complications was

highest in this group (1·4 per patient), and 10 patients died. A poor response to cortisone was obtained in 13 cases, in which an average daily dose of 50 mg. was given over a period of 19 months; this group suffered 1·2 complications per patient, and four patients died.

TABLE I

Response to Corticosteroid Therapy

TABLE Ia

Disease	Response			
	Good	Fair	Poor	Total
Systemic lupus erythematosus	10	12	2	24
Rheumatoid arthritis	5	6	2	13
Chronic hepatitis	3	6	2	11
Nephrosis	—	4	1	5
Miscellaneous	4	3	6	13
Total	22	31	13	66

TABLE Ib[1]

	Response			
	Good	Fair	Poor	Total
Number of complications	18	44	15	77
Number of complications per patient	0·8	1·4	1·2	1·2
Average duration (months)	32	31	19	30
Average total dose (grammes)	64	74	28	69
Average daily dose (mg.)	66	79	50	76
Ceased therapy	7 (30%)	5 (15%)	7 (55%)	19
Died	2 (9%)	10 (30%)	4 (30%)	16

[1] Fewer complications from corticosteroid therapy and fewer deaths occur in patients who show a good response to treatment with corticosteroids than in those who respond poorly.

TABLE Ic

Sex of Patient	Response			
	Good	Fair	Poor	Total
Males	8	12	2	22
Females	14	19	11	44

COMPLICATIONS

Age and Sex Distribution

The 22 males in the series received a greater average dose than did the 44 females, but they suffered the same incidence of complications (Table II). The incidence of complications was also similar in the 36 patients aged under 45 years to that in the 30 patients aged over 45, despite the fact that the older patients received more cortisone.

Individual Complications

Hypercorticism. — This syndrome was not evaluated as a complication. It occurred in minor degree in almost all cases of the series, but in only 30 was it noted as severe. The occurrence of severe hypercorticism was not associated with a more favourable response to treatment, or to an increased incidence of other complications.

Peptic Ulcer.—Fourteen patients suffered from dyspepsia during treatment; four of these had complained of indigestion before taking

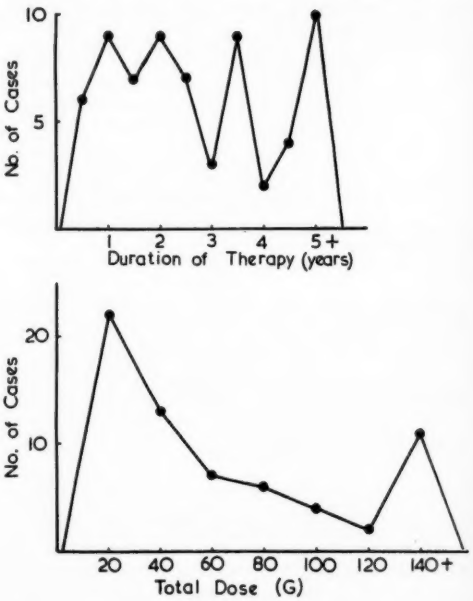

FIGURE I

The frequency distribution of the duration and total dose of corticosteroid therapy in the 66 cases

cortisone, and this became worse. A barium-meal X-ray examination was performed on eight of these patients, and showed three with ulcer (two were duodenal and one gastric). Eleven patients were investigated by histamine test meal. The two with duodenal ulcer showed a normal or high level of acidity (more than 60 units), as did two others. The patient with the gastric ulcer had a low gastric acidity (less than 40 units), as did six others. Four patients suffered hæmatemesis and melæna. Two required gastrectomy, one for persistent pain and a proven duodenal ulcer, and one for acute multiple gastric perforations and hæmorrhage.

Infection.—Twenty patients had infections during treatment; these included septicæmia

in two cases, pleurisy or pneumonia in 10 cases, abscess in five cases, tuberculous abscess in one case, and herpes zoster in one case.

Psychological Complications. — Twenty-one patients underwent some psychological change during treatment: four had frank psychotic episodes (hypomania in two, suicidal depression in two), and 17 had periods of pronounced euphoria or depression.

Osteoporosis.—Porosis of bone was not looked for as a routine procedure. Four patients sustained fractures: two were fractures of the forearm, one being due to a motor-car accident and the other to minimal trauma, while two were vertebral collapse.

TABLE II

Age and Sex Incidence of Complications[1]

	Age (Years)		Sex	
	Over 45	Under 45	Male	Female
Number of subjects ..	30	36	22	44
Average dose (grammes)	63	55	71	57
Therapy: average duration (months) ..	31	25	27	27
Average daily dose (mg.)	68	73	77	70
Number of side effects	37	40	24	53
Side effects per individual	1·2	1·1	1·1	1·2

[1] The incidence of complications from corticosteroid therapy is similar in both sexes and in both young and old age groups.

Adrenal Insufficiency.—Two of the 16 patients who died during the period of the study sustained only moderate trauma. In one case there was multiple bruising from a motor-car accident, and the other death followed reduction of a forearm fracture under general anæsthesia. The former patient received corticosteroid cover, but the latter did not. Another patient who suffered during life from chronic hepatitis died in unusual circumstances at home after having taken a total of 30 grammes of cortisone over a period of eight months. Autopsy failed to reveal an adequate cause for death, and she probably died from adrenal insufficiency.

Pancreatitis.—Two patients with systemic lupus erythematosus developed pancreatitis while receiving corticosteroids.

Hypertension.—This complication was uncommon, since in only two cases was it considered that a significant rise in arterial pressure was caused by corticosteroids alone. One patient was a man, aged 59 years, with nephrotic syndrome, whose arterial pressure rose steadily after the beginning of treatment from 160/100 to 220/130 mm. Hg, at which stage hypotensive drugs were given. When the corticosteroid was discontinued, the hypertension also regressed. The other patient was a man, aged 49 years, with cardiomyopathy, whose arterial pressure rose under treatment from 100/70 to 180/120 mm. Hg. He was not treated with hypotensive drugs.

Glycosuria.—Routine urine tests gave negative results for glucose with the exception of two cases. A girl, aged 21 years, with systemic lupus erythematosus, showed pronounced glycosuria after cortisone therapy was commenced, and this disappeared when the treatment was discontinued. A woman, aged 54 years, with ulcerative colitis, who had been observed for several years and had never been noted to have glycosuria, died in acute diabetic coma. Glucose tolerance tests were not done as a routine procedure.

Bruising.—Purpura or a tendency to bruise on minimal trauma was noted in eight cases.

Fluid Retention.—Since œdema is a frequent symptom in systemic lupus erythematosus, hepatitis and the nephrotic syndrome, fluid retention was assessed as a complication only in rheumatoid arthritis and the miscellaneous diseases. Two patients with rheumatoid arthritis developed œdema while receiving corticosteroids, and one of these repeatedly developed congestive cardiac failure when receiving high doses.

THE RELATION BETWEEN DISEASE, AMOUNT OF THERAPY AND COMPLICATIONS

Certain diseases were more often associated with specific complications than others (Table III). Patients with systemic lupus erythematotus had a high incidence of psychological complications and bruising, while patients with rheumatoid arthritis showed the greatest frequency of symptoms suggesting peptic ulcer. The group with rheumatoid arthritis had the highest rate of complications per patient, and their average age was highest. There were fewest complications in the group with active chronic hepatitis, even though they received almost as much corticosteroid as the group with rheumatoid arthritis and had a much higher daily dose.

Some indication of the influence of duration and dose of therapy on the appearance of complications can be gauged by relating the mean number of complications per patient in groups receiving different amounts of corticosteroid over different periods of time. Figure II shows that as both duration and dose increased, so did the average number of complications, from less than one per patient to more than two per patient.

TABLE III

Disease and Incidence of Complications[1]

TABLE IIIA

Complication	Systemic Lupus Erythematosus	Rheumatoid Arthritis	Chronic Hepatitis	Nephrosis	Miscellaneous	Total
Peptic ulcer	6	7	1	1	—	14
Infection	8	6	3	1	2	20
Psychological upset	11	3	2	2	3	21
Osteoporosis	—	2	1	1	—	4
Adrenal insufficiency	1	1	—	—	—	2
Pancreatitis	2	—	—	—	—	2
Hypertension	—	—	—	1	1	2
Diabetes	1	—	—	—	1	2
Fluid retention	—	2	—	—	—	2
Bruising	5	—	—	1	2	8
Total	34	21	7	7	9	77

TABLE IIIB

—	Systemic Lupus Erythematosus	Rheumatoid Arthritis.	Chronic Hepatitis	Nephrosis	Miscellaneous	Total
Number of patients	24	13	11	5	13	66
Number of complications per patient	1·4	1·6	0·6	1·4	0·7	1·2
Average age (years)	38	55	40	41	39	44
Average duration (months)	37	36	27	22	16	30
Average dose (grammes)	90	80	76	49	22	69
Average daily dose (mg.)	80	74	93	74	45	76

[1] This limited study suggests that the nature of the disease treated influences the nature of the complications which result from corticosteroid therapy.

CEASING THERAPY

Nineteen of the 66 patients ceased therapy. Ten were able to cease because of remission of their disease, and in five cases corticosteroids were discontinued because it became apparent after an extended trial that they were not influencing the course of the disease. Complications induced by corticosteroids caused cessation of treatment in only four cases; two of these were psychotic episodes, one was gross hypercorticism, and one was pancreatitis. In eight cases attempts were made to cease corticosteroids because the disease treated was regarded as inactive, but return of symptoms necessitated recommencement of treatment.

DEATH DURING THERAPY

Sixteen of the treated patients died. Of these, 12 died from their disease; in four cases corticosteroids were thought to be associated with the cause of death. Two died hypotensive deaths after accidental or surgical trauma, and one died from multiple gastric perforations and hæmorrhage. A further patient died in unusual circumstances at home.

DISCUSSION

Benefits derived from corticosteroids in many diseases are undoubted, but the incidence and severity of complications have caused concern. The value of the present study lies in the information it gives about the relative importance of the factors which influence the occurrence of complications in corticosteroid therapy. Age and sex have no effect, but the type of disease treated has a considerable influence on the nature and frequency of the complications which result from cortisone therapy. More complications occur as the dose of cortisone and the period during which it is taken increase.

A point not brought out in other series is the prognostic significance of the initial response to corticosteroid therapy. Those patients who responded with marked improvement to corticosteroids suffered fewest complications, though they received treatment for the longest period. The initial response to corticosteroids is partly a measure of the severity and stage of the disease treated, but a good response appears to carry a good prognosis as regards both mortality and the hazards of treatment. Conversely, those who experienced no benefit from corticosteroids received only small doses, yet incurred more complications per person than the first group, so that a poor response appears to carry a poor prognosis in this regard.

The greatest number of complications occurred in the group of patients who responded only moderately to corticosteroids. Though their response was indifferent, it was apparently

sufficiently encouraging to maintain treatment, and the average daily dose is highest in this group. Mortality is of the same order (30%) as in the group who showed no improvement, though the severity of disease in this moderate response group is less. Only 15% were able to cease therapy. It is in these cases that the chronic discomfort of poorly-controlled disease encourages the physician to increase the doses of corticosteroids and usher in more hazardous complications.

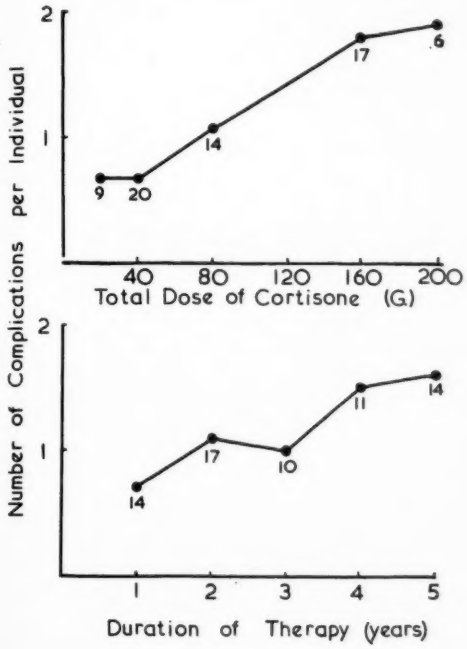

FIGURE II

There is a greater average number of complications per individual patient as the dose and duration of corticosteroid therapy increase. Small figures indicate the number of patients in each group

Since prognosis is more encouraging in patients who show an immediate favourable response to corticosteroids, it follows that in any survey the proportion of patients who respond well will influence the conclusions made about the value and hazards of that treatment. Boland (1951) reported that there was a much more favourable response to corticosteroids (75% "dramatic") in patients with only moderate disease, and that only 18% of this group suffered from adverse side effects. However, of patients with severe disease, 64% had a response classed as "good" rather than "dramatic", and there was a 56% incidence of side-effects. Bunim *et alii* (1955) presented 71 cases of rheumatoid arthritis, in most of which there was only minor benefit from prolonged therapy. He concluded that apparent benefit from corticosteroids was influenced by the proportion of patients who had suffered from their disease for less than a year, were young, and had active disease and reversible joint disorder. The greater incidence of complications in patients with severe disease is due in part to the difficulty in deciding whether a particular symptom is caused by the disease or the treatment. We have already noted this difficulty with fluid retention and hypertension, and it will be noted also with other complications as they are discussed in relation to other series.

Effect of Disease, Dose, Age and Sex on Complications

The type of disease treated seems to be as important as the amount of corticosteroid in determining the incidence of complications. The 11 patients of this series who suffered from chronic hepatitis incurred few complications, even though they received the highest daily dose of cortisone. Goldgraber and Kirsner (1959) comment on the notable lack of adverse effects from corticosteroids in patients treated for liver disease.

The average number of all complications per patient rose gradually with both increased duration and increased total doses of corticosteroids, and the findings do not suggest that only above a certain period of treatment are certain complications imminent. No total dose or period is "safe", and none will inevitably produce a particular undesirable side effect; however, as treatment is prolonged, each hazard mentioned becomes gradually more likely. This apparent lack of a threshold effect may be due only to the relatively crude technique used to demonstrate it. As the daily dosage of corticosteroid is varied with the fluctuating course of these chronic diseases, there may be for each case a threshold of daily dosage above which a particular complication is more likely to occur. Bunim *et alii* (1955) always kept their maintenance dose of cortisone below 100 mg. per day, after their initial experience that three patients who exceeded that schedule all developed peptic ulcers, two of which perforated.

Boland (1951), in his series of 76 patients with rheumatoid arthritis, noted that side effects from corticosteroids were more common in women. This is not confirmed by the present study, in which the sex incidence of complica-

tions is equal. Neither can we agree with the statement of Kern (1957) that "all the bad effects of corticosteroid therapy occur with far greater frequency in those less and less able to cope with them—those over the age of 50", as the incidence of complications in the present series is the same in old and young.

Individual Complications

The incidence of particular complications in this series is consistent with that in other reports of cases of prolonged corticosteroid therapy.

Dyspepsia.—Dyspepsia occurred most frequently in the patients with rheumatoid arthritis (seven out of 13), though only three had proven ulcers. Kammerer *et alii* (1958) reported radiological evidence of ulcer in 36 out of 117 patients with rheumatoid arthritis receiving corticosteroids. Savage (1959), on the other hand, found a demonstrable ulcer in only 7% of 910 patients with rheumatoid arthritis receiving corticosteroids. West (1959) reduced dyspepsia in his patients by using enteric-coated prednisolone tablets, and suggested that the ulcergenic action of the drug was local, akin to that of aspirin. However, he pointed out that nearly all patients with rheumatoid arthritis also took aspirin, and that peptic ulceration occurred in that disease before the introduction of corticosteroids.

Psychological Change.—Psychological change was most frequently observed in patients with systemic lupus erythematosus. However, both psychoneurotic reactions and psychoses occur in systemic lupus erythematosus not treated with corticosteroids. O'Connor (1959) concluded that diffuse brain damage resulting from the disease, and innate personality changes, were just as important as corticosteroids in determining the onset of a psychotic episode in this disease.

Infection.—Masked infection has frequently been reported as a cause of death of patients receiving corticosteroids. Burrage *et alii* (1955) cited the case of an asthmatic patient receiving cortisone who survived a duodenal ulcer and vertebral collapse, but died suddenly and unexpectedly of unsuspected bilateral pneumonia. Although a number of severe infections, including two cases of septicæmia, occurred in the present series, none was fatal. A tuberculous ischiorectal abscess appeared in one case; but in three patients who had previously been treated for tuberculosis there was no exacerbation of the infection, probably because of prophylactic antituberculosis chemotherapy administered concurrently with corticosteroids.

Osteoporosis.—Osteoporosis occurs in association with immobility in rheumatoid arthritis, as reported by Baer (1941), who described a series of patients with fractures. Copeman *et alii* (1954), reporting on 20 patients with rheumatoid arthritis treated for one to three years with cortisone, found that, though their patients felt improved, eroded bone was not repaired, and in eight cases the radiological appearances continued to deteriorate. On the other hand, the Medical Research Council and Nuffield Foundation Joint Committee (1959) found that after two years a group of rheumatoid patients treated with prednisolone showed no progression of bony changes in hands and feet, while a control group receiving only aspirin showed increased erosive changes. The consequences of bony softening are usually unheralded, and the most serious is vertebral collapse. Demartini *et alii* (1952) considered that this particular lesion had become much more frequent since the introduction of corticosteroids, as it was rare in Baer's 1941 series. Burrage *et alii* (1955) reported 30 cases of allergic disorders treated with cortisone for periods of from one to four years. Four of these patients suffered vertebral collapse, one after only 65 days' therapy.

Adrenal Atrophy.—Kern (1957) maintained that three months of corticosteroid therapy would cause adrenal atrophy. He demonstrated an increase in the mortality of older patients with asthma during the years that cortisone came into general use, and associated this with an increase in the number of "strange" deaths (similar to the one mentioned in this series), blaming corticosteroid. Larzelere *et alii* (1954) investigated adrenal atrophy in long-term corticosteroid therapy by the response to ACTH infusions in 22 male patients, by estimating urinary excretion of 17-ketosteroids and free corticoids, and by eosinophil counts. In 19 cases responsiveness was delayed; but these workers were unable to correlate this delay in any way with the amount or duration of corticosteroid treatment.

Diabetogenic Effect.—The diabetogenic effect of corticosteroids is well recognized (Bunim *et alii*, 1952). Sash (1959) reported the case of a man, aged 71 years, with rheumatoid arthritis, maintained on doses varying between 37·5 and 75 mg. of cortisone daily, who presented in diabetic coma. Autopsy revealed fat necrosis typical of acute pancreatitis. One patient of our series died in diabetic coma; the pancreas at autopsy showed no macroscopic evidence of inflammation, but histological examination revealed multiple microscopic abscesses. Carone and Liebow (1957), reviewing

54 consecutive autopsies on patients who had received cortisone therapy, found 16 with evidence of pancreatitis. This finding, and other recent reports of pancreatitis during corticosteroid therapy, suggest that the pancreatitis which occurred in two cases of systemic lupus erythematosus in the present series was caused by the corticosteroids.

CONCLUSION

This survey suggests that a certain incidence of complications from corticosteroid therapy is unavoidable, and that complications are usually unpredictable. A regular careful search for incipient complications by objective assessment of psychological state and dyspeptic symptoms, radiological examination for osteoporosis and dyspepsia, and measurement of blood pressure, serum amylase content and glucose tolerance, should allow prediction and reduction of side-effects. However, we still require more evidence that this is so, and it is hoped that further surveys will clarify this problem.

ACKNOWLEDGEMENTS

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DISSEMINATED TUBERCULOSIS AND BONE MARROW DYSCRASIAS[1]

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SUMMARY

The clinical features and autopsy findings are described in two cases of pancytopenia, four cases of leukæmia and one case of myelofibrosis, all of which were associated with disseminated tuberculosis.

It is considered that tuberculosis preceded and may have caused pancytopenia in three cases. Diagnosis in such cases is aided by biopsy of lymph glands, liver and bone marrow from the iliac crest, and by culture of biopsy material. The pancytopenia should respond to anti-tuberculous chemotherapy, but in one case it progressed to leukæmia despite treatment.

Tuberculosis appeared as a complication in three cases of leukæmia and in one case of myelofibrosis. These cases pursue their natural course despite anti-tuberculous chemotherapy. Tuberculosis seems more likely to occur in patients receiving cortisone.

We consider that "leukæmoid reaction" to tuberculosis is not common, and that, more commonly, leukæmia is complicated by tuberculosis. In a few cases tuberculosis may be an ætiological factor in the blood dyscrasia, most commonly in cases of pancytopenia.

THE association between disseminated tuberculosis and hæmatological disorders was recognized by Volpe (1896), by Osler (1907) and by Parkes Weber and Schluter (1936). Most reports referred to changes in the myeloid series. More recently other blood dyscrasias have been described, such as hypoplastic anæmia (Kernohan, 1950), monocytic leukæmoid reaction (Gibson, 1946), lymphocytic leukæmoid reaction (Gardner and Mettier, 1949; Staffurth and Spencer, 1950), myelofibrosis (Crail *et alii*, 1948), polycythæmia (Guild and Robson, 1950) and anæmia due to hypersplenism from tuberculous splenomegaly (Engelbroth-Holm, 1938). Such cases show unusual features and present problems in diagnosis. We have reviewed seven cases in which blood dyscrasias were associated with miliary tuberculosis at the Royal Melbourne Hospital; of these, there were four cases of leukæmia, two of pancytopenia and one of myelofibrosis.

A study of these cases suggests that disseminated tuberculosis was possibly associated with the development of pancytopenia and marrow hypoplasia in two patients. In at least two cases of leukæmia and the case of myelofibrosis, the tuberculosis appears to have been a terminal condition, its development being favoured by debility or by cortisone therapy.

The term "leukæmoid reaction" requires definition. By it we mean the presence of large numbers of white cells with immature forms in the peripheral blood, without gross anæmia or thrombocytopenia, secondary to some non-leukæmic disease process. Changes in the marrow are limited to hypercellularity, and hæmatological changes return to normal if the underlying condition is satisfactorily treated. We consider a marrow count of over 20% of blast cells in a hypercellular marrow to indicate leukæmia.

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REPORTS OF CASES

In Cases I and II pancytopenia and tuberculosis were associated.

CASE I (201004).—In 1959, a clerk, aged 55 years, developed a respiratory infection and remained in poor health for four months. No antibiotics were prescribed, and he had no contact with chemicals. He then developed sores in his mouth and a rash. Examination showed pyrexia (a temperature of 39° C.), tachycardia (a rate of 120 per minute) and widespread crepitations and rhonchi throughout the chest. The liver, spleen and lymph nodes were not enlarged. X-ray examination of the chest showed linear scarring in the right mid-zone and increased density in the right hilar region. The hæmoglobin value was 7·4 grammes per 100 ml., the leucocyte count 3000 per cubic millimetre, and the platelet count 100,000 per cubic millimetre. The bone marrow was hypocellular; erythropoiesis was normoblastic with marked rouleaux formation. Granulocytes and megakaryocytes were reduced in number, and there was an increase in lymphocytes of varying size and nuclear shape. The patient improved with blood transfusion and erythromycin, but his temperature did not fall and the X-ray appearances in his chest were unchanged. Two weeks later he developed ulcerative pharyngitis. A chest film showed an apparent extension of the hilar opacity. He died suddenly.

At autopsy, there were large caseous hilar glands and the lower lobes of both lungs were consolidated. Tubercles were present beneath the bronchial mucosa

and throughout the lungs, liver and spleen. Microscopic examination also showed miliary tubercles in the bone marrow, which was otherwise normal. The tubercles showed extensive necrosis, but minimal cell accumulation and very few giant cells (Figure I). Both lungs also showed a necrotizing granulomatous pneumonia. Acid-fast bacilli were readily demonstrated.

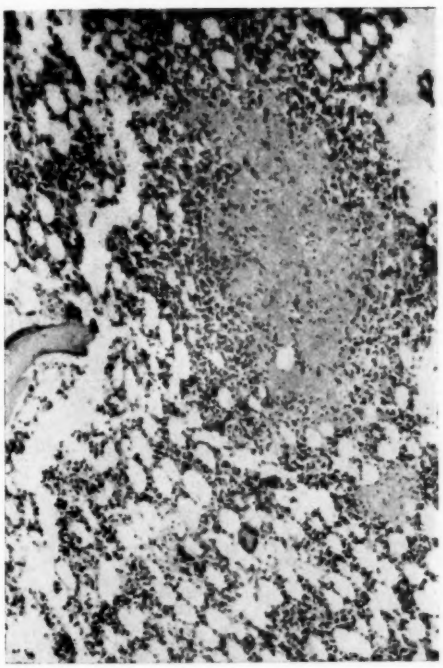

FIGURE I

Case I: pancytopenia and tuberculosis. Typical tubercle in bone marrow shows necrotic centre with minimal inflammatory cell accumulation. (Hæmatoxylin and eosin. ×65)

The clinical features suggest pulmonary tuberculosis of four to five months' duration. Lymphoma or infection was suspected as a cause for pancytopenia, but not proven, and no cultures were attempted. Tubercles showing dense necrosis, numerous acid-fast bacilli and little inflammatory reaction suggest a heavy infection of short duration.

CASE II (150819).—A woman, aged 64 years, presented in 1953 with four months' history of weakness and unexplained pyrexia. The spleen was palpable 3 cm. below the rib margin and was tender. There was pancytopenia, the hæmoglobin value being 7·4 grammes per 100 ml., the leucocyte count 1200 per cubic millimetre with a relative lymphocytosis and the platelet count 70,000 per cubic millimetre. The sternal marrow was hypoplastic, with increased numbers of small lymphocytes. A high titre to *Salmonella typhi* O antigen (1 : 2560) was found, possibly related to recent vaccination. The titre of *S. typhi* H was 1 : 40 and of *S. paratyphi* A and B 1 : 20. The patient was treated with chloramphenicol and blood transfusion. Later, examination of marrow from the iliac crest revealed small foci of epithelioid cells. A liver biopsy also showed granulomas with epithelioid cells and giant cells, but neither necrosis nor acid-fast bacilli. Salmonella agglutination titres fell to normal levels, although pyrexia persisted. Repeated examinations of the bone marrow, with cultures, were unrewarding. Blood transfusions were required every six weeks. Pulmonary opacities appeared, and she developed bronchopneumonia before death, a year after presentation.

At autopsy, the spleen was enlarged (400 grammes). Necrotic granulomas were present in the mediastinal glands, liver and kidney. There were epithelioid cells and occasional giant cells, but no acid-fast bacilli. The marrow was hypoplastic, but contained no tubercles.

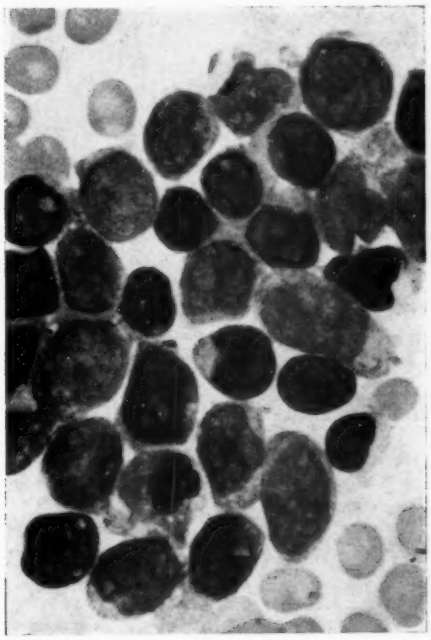

FIGURE II

Case III: patient presented with pancytopenia and later tuberculosis. Despite antituberculous therapy, leukæmia developed. Sternal marrow during terminal phase, showing sheets of myeloblasts. (Leishman. ×1200)

Presentation with fever and raised typhoid agglutination titres has been described (Medd and Hayhoe, 1955). An early onset of tuberculosis is suggested by the small granulomas found on biopsy. The classical tubercles found at autopsy a year later are consistent with the insidious progress of the disease in this case.

Case III was one of pancytopenia, tuberculosis and leukæmia.

CASE III (184037).—A Japanese girl, aged 26 years, presented herself in 1958 with intermittent pyrexia and symptoms of anæmia. She was 12 miles from Hiroshima in 1945, but it was considered that she had received negligible irradiation. An X-ray examination of the chest showed enlarged mediastinal glands, and although the result of the Mantoux test was strongly positive, no acid-fast bacilli were cultured from the sputum. There was pancytopenia, the hæmoglobin value was 8·5 grammes per 100 ml., the leucocyte count was 750 per cubic millimetre, and the platelet count was 98,000 per cubic millimetre. The differential leucocyte count showed an almost total absence of polymorphs, with a pronounced relative lymphocytosis. The bone marrow was very hypocellular. The ratio of normoblasts to leucocytes was 1 : 11 ; there were few megakaryocytes, but increased lymphocytes (30%) and immature granulocytes (myeloblasts 28%). Marrow films showed neither granulomas nor acid-fast bacilli. With blood transfusion and cortisone there appeared to be almost complete marrow regeneration over the next month. This suggested a toxic depression of the marrow. After two months, cortisone therapy was stopped.

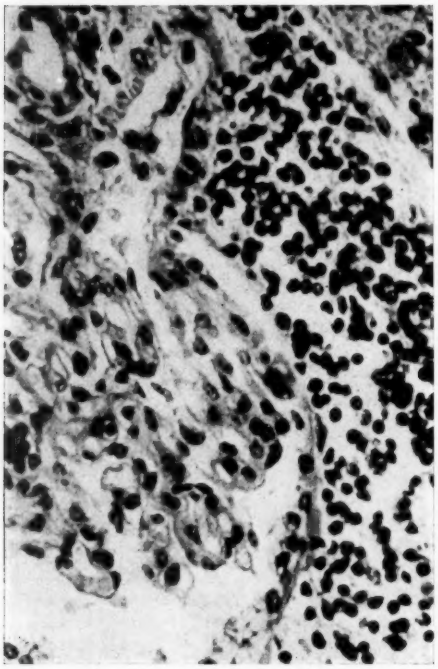

FIGURE III

Case III : despite hypercellular marrow with over 90% myeloblasts (Figure II), autopsy revealed little leukæmic infiltration of organs. Kidney, showing minimal infiltration with leukæmic cells about a glomerulus. (Hæmatoxylin and eosin. ×480)

Seven months later opacities were seen in the left lung on X-ray examination, and the patient developed several enlarged cervical glands. Biopsy of one gland showed tissue necrosis and numerous acid-fast bacilli. The biopsy wound continued to discharge, and culture of this fluid yielded *Mycobacterium tuberculosis*. Antituberculous therapy was commenced. However, five months later she developed subleukæmic leukæmia. The leucocyte count was 1400 per cubic millimetre, 2% being myeloblasts. The bone marrow was very cellular, and examination showed 66% myeloblasts. Neutropenia and anæmia persisted and she required regular blood transfusions. Finally her marrow was found to contain nearly 100% myeloblasts (Figure II). She developed severe cellulitis and died two years after presentation.

At autopsy, the apices of both lungs showed fibrosis and caseation ; the Fallopian tubes contained caseous material. Red bone marrow extended into the lower part of the femur. The liver weighed 1830 grammes and the spleen 150 grammes. Microscopic examination revealed caseous necrosis in the lung and Fallopian tubes, surrounded by granulation tissue and a few lymphocytes. No acid-fast bacilli were seen. Minimal leukæmic infiltration was present in the adrenal glands, liver and kidney (Figure III).

In this case there is progression from marrow hypoplasia to leukæmia—a sequence of events seen in benzene poisoning. Tuberculosis appears to have been present from the onset of her illness and may have caused the marrow hypoplasia. However, leukæmia developed during antituberculous chemotherapy, and the marrow hypoplasia may merely represent a phase in the leukæmic process, the underlying cause of which is obscure. Autopsy findings suggest inactive

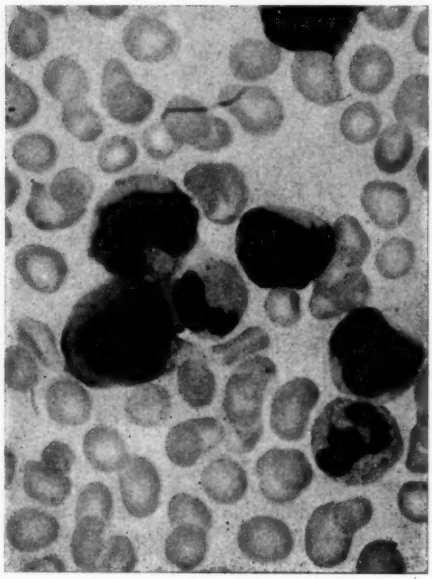

FIGURE IV

Case IV : acute myeloid leukæmia. Blood smear at early stage, showing myeloblasts (50%), including some with a monocytoid form. (Leishman. ×1200)

tuberculosis and leukæmia with minimal organ infiltration.

In Cases IV to VI leukæmia and tuberculosis were associated.

CASE IV (190525).—A woman, aged 24 years, was admitted to hospital in 1959, three months after curettage for menorrhagia. She complained of tiredness, purpura and epigastric discomfort. Examination of the patient showed pallor and petechiæ of the skin and oral mucosa. The temperature was 38 °C. and the liver and spleen were greatly enlarged. The hæmoglobin value was 5·7 grammes per 100 ml., and the leucocyte count 30,000 per cubic millimetre, of which 50% were myeloblasts (Figure IV). The platelet count was 52,000 per cubic millimetre. The bone marrow was cellular, with normoblastic erythropoiesis. Examination of the white-cell series showed 19% myeloblasts and 39% promyelocytes. Megakaryocytes were reduced in number. X-ray examination of the chest revealed no abnormality.

Blood transfusion and 6-mercaptopurine (6-M.P.) produced improvement for about three months. Pyrexia persisted and her anæmia returned. She was given prednisolone, further 6-M.P. and blood transfusion, but her condition deteriorated. The leucocyte count fell steadily, even after cessation of therapy.

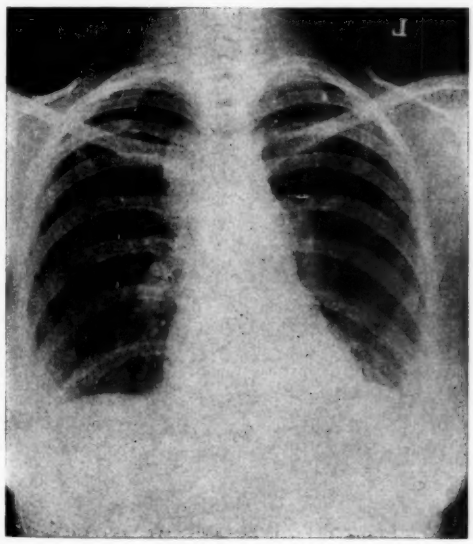

FIGURE V

Case IV: acute myeloid leukæmia, initial chest X-ray appearances normal. Chest symptoms developed a week before death, when chest X-ray film (above) showed enlarged hilar shadows and mottling of lung fields

A week before death she developed severe chest pain and a high fever. X-ray examination of the chest showed enlarged hilar shadows and generalized mottling of the lung fields (Figure V).

At autopsy, large yellow necrotic glands were present in the mediastinum, and the lungs and spleen showed miliary nodules. Petechial hæmorrhages were present in the heart, stomach and brain. The lower one-third of the femur contained red bone marrow. The weights of the liver and spleen were 2775 grammes and 360 grammes respectively. Microscopic examination revealed intensive necrotizing pneumonia. The lungs, liver, spleen, thyroid, heart and stomach all showed foci of eosinophilic necrosis surrounded by a few lymphocytes and containing numerous acid-fast bacilli (Figure VI). There was no leukæmic infiltration of liver of spleen.

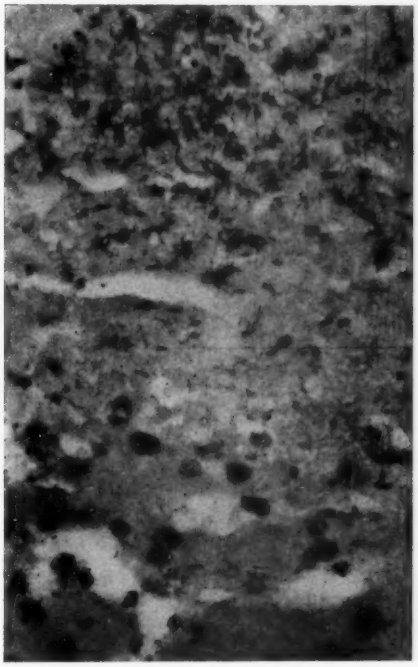

FIGURE VI

Case IV: acute myeloid leukæmia. Vast numbers of acid-fast bacilli in tubercles suggest fulminating infection of short duration. Edge of necrotic focus in liver showing absence of inflammatory cell accumulation and numerous acid-fast bacilli. (Ziehl Neelsen. ×480)

The late development of respiratory symptoms and radiological signs suggests that tuberculosis occurred terminally in leukæmia. The microscopic appearances of the tubercles are consistent with a recent massive inoculation with *M. tuberculosis*. The lack of leukæmic infiltration may be due to drug therapy which caused a pronounced leucopenia.

CASE V (102637).—In 1947, a man, aged 61 years, was admitted to hospital for investigation of anæmia. For three months he had noticed tiredness, anorexia and weight loss and, later, pallor. He had a cough with yellow sputum. Many rhonchi and râles were present in his chest, although X-ray examination revealed no abnormality. His temperature was 39° C. The liver and spleen were not enlarged. The hæmoglobin value was 7·9 grammes per 100 ml., and the leucocyte count

was 1300 per cubic millimetre. Very few platelets were seen on the films. The differential leucocyte count showed a slight shift to the left of neutrophils, but no blast cells, and a slight relative lymphocytosis. The bone marrow was cellular, most cells (77%) being myeloblasts. The patient's condition deteriorated, and he developed a purpuric rash and died within a week.

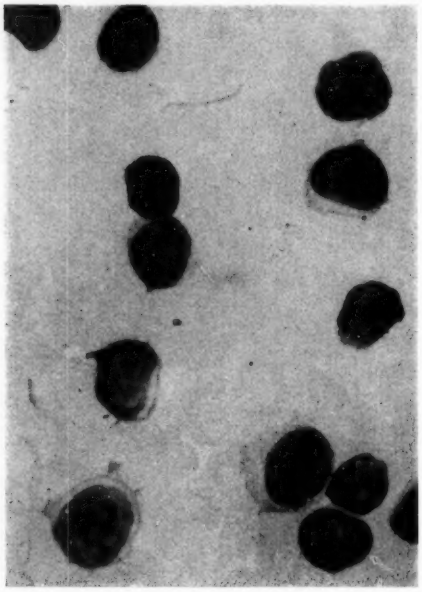

FIGURE VII

Case VI: lymphocytic leukæmia. Blood smear, showing numerous mature small lymphocytes. (Leishman. ×1200)

At autopsy, lower lobe consolidation was found in both lungs, and there were ecchymoses throughout the bowel. Microscopic examination of sections showed characteristic necrotic tubercles in the liver, spleen and lymph nodes, with few inflammatory cells or giant cells. There was no evidence of leukæmic infiltration of organs. The appearance of the bone marrow at autopsy was not recorded.

The presence of 77% of myeloblasts in the marrow indicates leukæmia, despite negative autopsy findings. No specific therapy was given, so that drugs cannot account for the lack of leukæmic infiltration. The granulomas are typical of a fulminating tuberculous infection which may have cut short the leukæmia.

CASE VI (177515).—A man, aged 65 years, was examined in 1957 because of increasing tiredness for three years. He had been treated for chronic lymphatic leukæmia with blood transfusion and triethylene melamine for five months. Indurated areas were present on the face, and cervical lymph glands were enlarged. Biopsy of both these lesions showed extensive lymphocytic infiltration. The hæmoglobin value was 9·0 grammes per 100 ml., the leucocyte count was 390,000 per cubic millimetre, and 98% of cells were lymphocytes (Figure VII). Few platelets were present on the blood film. In the bone marrow almost all the cells were mature lymphocytes. Further triethylene melamine was given; he died ten weeks later.

At autopsy there was moderate leukæmic infiltration of hilar and mesenteric lymph nodes, the liver, kidneys and adrenal glands. These changes were not so striking as those seen in the previous skin biopsy. Areas of eosinophilic necrosis were found in the lung, liver and spleen, around which were epithelioid cells and lymphocytes, occasional giant cells and numerous acid-fast bacilli.

Leukæmic infiltration appeared to decrease as the illness progressed, perhaps owing to triethylene melamine therapy. Tuberculosis was unsuspected before death.

Case VII was one of myelofibrosis complicated by tuberculosis.

CASE VII (178138).—A man, aged 71 years, noticed bruising and an abdominal mass in 1957, and complained of tiredness and shortness of breath. The spleen was greatly enlarged, and there was extensive purpura. The temperature and erythrocyte sedimentation rate were normal. Blood examination showed a small proportion of immature red and white cells. The hæmoglobin value was 11·1 grammes per 100 ml., the leucocyte count 8000 per cubic millimetre. Bone marrow from the iliac crest showed considerable dense fibrosis with a reduction in hæmatopoietic elements.

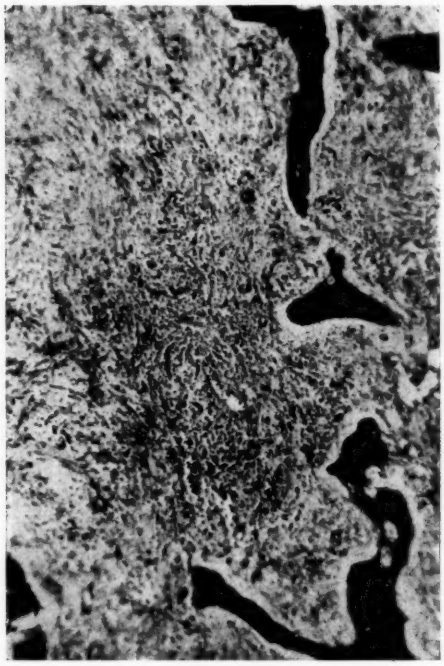

FIGURE VIII

Case VII: bone marrow showing myelofibrosis with remaining islets of hæmatopoietic cells. (Hæmatoxylin and eosin. ×65)

There was improvement with blood transfusion, but two months later he developed a cough and noticed increasing weakness. His condition deteriorated and he died.

At autopsy, the lower lobes of the lungs were consolidated and there were numerous yellow nodules throughout the spleen. Microscopic examination of sections showed areas of eosinophilic necrosis in the liver, spleen and bone marrow, surrounded by lymphocytes and occasional giant cells. The lungs showed no evidence of tuberculosis. The bone marrow showed patchy replacement by fibrous tissue (Figure VIII), but elsewhere was quite cellular, with some immature cells. The sinusoids of the liver and spleen contained mature leucocytes. Acid-fast bacilli were demonstrated in the liver.

Chest infection developed after the onset of myelofibrosis. There was no pyrexia or lymphadenopathy, as is described by Crail, Alt and Nadler (1948) in cases of myelofibrosis attributed to miliary tuberculosis, and this patient is older than the patients they recorded.

DISCUSSION

Most chronic infections affect the bone marrow (Cartwright and Wintrobe, 1952), causing maturation arrest of erythropoiesis, altered iron utilization and hæmoglobin synthesis. Different infections cause consistent and rather characteristic changes in the proportion of lymphocytes or polymorphonuclear leucocytes in the peripheral blood. The changes in experimental tuberculous infection are striking. "Leukæmoid reactions" have been produced in tuberculous rabbits and calves by the injection of tuberculin (Feldman and Stasney, 1937; Stasney and Feldman, 1938). Non-tuberculous rabbits were not affected by tuberculin, whereas tuberculous animals developed leucocyte counts of 100,000 per cubic millimetre. It is reasonable to suppose that circulating tuberculin in infected humans may produce similar effects. Leucocytes in cases of advanced tuberculosis may resemble leukæmic leucocytes serologically (Schwartz *et alii*, 1955).

There are several ways in which disseminated tuberculosis could cause pancytopenia. In our cases hypersplenism (Kernohan, 1950; Engelbroth-Holm, 1938) and direct involvement of the bone marrow by tuberculosis (Ball, Joules and Pagel, 1951) were minimal. Tuberculin can cause local tissue necrosis in the skin of sensitized subjects. The production of large amounts of tuberculin in disseminated tuberculosis may cause necrosis or maturation arrest of marrow cells if they are particularly sensitive. Granulomas in the bone marrow do not appear to be essential for this type of change; however, this theory lacks experimental proof.

The histological features of tuberculosis in these conditions are characteristic, in that there is extensive necrosis with little inflammatory cell accumulation. This may be due to an alteration in tissue response, perhaps conditioned by an underlying severe illness or by certain drugs. An altered tissue response is indicated by an unusually high rise in titre to *S. typhi* O antigen, seen in Case II and other reported cases (Medd and Hayhoe, 1955). On the other hand, the same authors suggest that a sudden massive dose of organisms, causing extensive miliary tuberculosis, may also produce these necrotic tubercles without cell accumulation. The large numbers of organisms seen in histological sections (Figure VI) would support this. Probably both factors play a part.

The absence of leukæmic infiltration of organs is unusual, and has often been accepted as proof that these processes are "leukæmoid reactions" (Hughes, Johnstone, Scott and Stewart, 1959). On the other hand, Rosenthal (1956) suggested that, in his and most other cases, the leukæmia had been cut short by incidental miliary tuberculosis. If this occurs at a very early stage, the clinical and hæmatological picture will be poorly developed and leukæmic infiltration of organs may be slight. This may have occurred in Case V. Cytotoxic drugs cause leucopenia, as in Case IV, and probably diminish leukæmic infiltration of organs. These drugs and corticosteroids may be important in provoking dissemination of tuberculosis terminally in patients with debilitating diseases (D'Arcy Hart and Rees, 1950; Mackinnon, 1959; Iverson and Ofstadt, 1960).

Multiple criteria are used to distinguish between leukæmia and "leukæmoid reactions" (de Gruchy, 1958). These include clinical, laboratory and autopsy findings and the response to treatment of an underlying condition. We consider that, in our four cases, the clinical and laboratory evidence for leukæmia outweighs the absence of significant leukæmic infiltration of organs at autopsy. Such cases would be expected to progress fatally in spite of antituberculous therapy, as did those described by Rosenthal (1956). They should not be regarded as "leukæmoid reactions". If the incidence of leukæmia increases and death from tuberculosis decreases, the diagnosis of "leukæmoid reaction" will require critical assessment.

While there is experimental evidence that tuberculosis causes changes in the blood and bone marrow, chance association between tuberculosis and blood dyscrasias is also to be expected. In our cases of pancytopenia, clinical findings suggest that tuberculosis was present throughout the illness and was perhaps responsible for the hæmatological changes. However, pancytopenia may have progressed to leukæmia had the patients survived longer,

and the occurrence of tuberculosis may be fortuitous. In Case III pancytopenia did progress to leukæmia. In three cases of leukæmia and one of myelofibrosis, tuberculosis occurred terminally. This complication may be favoured by the use of cytotoxic drugs or corticosteroids.

The presence of fever or respiratory symptoms in patients with blood dyscrasias should arouse the suspicion of tuberculosis. The diagnosis can be made only by histological examination of sections and culture of affected tissues, such as liver, lymph nodes and bone marrow. Both biopsy and culture may need to be repeated on several occasions. In cases of pancytopenia, tuberculosis may be responsible for the hæmatological changes. If this is so, they may be expected to respond to antituberculous therapy. Progression to leukæmia in such cases, despite antituberculous treatment, may have two explanations. First, there may be a fortuitous association between tuberculosis and a leukæmic process, the latter being manifest at an early stage by pancytopenia. Secondly, products of the tubercle bacillus may cause severe depression of the bone marrow, and this may be irreversible and initiate leukæmia. Such an effect is thought to occur with benzene and phenylbutazone (Whitby and Britton, 1957; Bean, 1960). In other cases tuberculosis is clearly a terminal event, complicating an established and unrelated blood condition, and perhaps accentuated by cortisone and cytotoxic agents.

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A Review Written by Request

FAT ABSORPTION

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AUTHORITATIVE reviews of fat absorption have been written both for laboratory workers and for clinicians during the last few years by leaders in the field (for example, Bergström and Borgström, 1955; Deuel, 1955; Frazer, 1958). Annual reviews help to place recent contributions in perspective (Hogben, 1960). With such guides available, another detailed review would be out of place. This article presents only an outline of the subject as seen by a non-biochemist who strayed into this highly chemical field through an interest in intestinal lymph. Attention will be concentrated on the absorption of triglycerides, which are the predominant type of fat in the diet. For ease of discussion, absorption will be considered mainly in successive stages: entry of fat into the epithelial cell, changes undergone during passage through the cell and, finally, removal of the absorbed material from the intestinal tissue fluid. This distinction cannot always be maintained, since the stages at which some experimental procedure or clinical abnormality has affected absorption cannot always be determined from the results.

ENTRY OF FAT INTO THE EPITHELIAL CELL

Long-Chain Fats

In the common dietary fats the triglycerides of long-chain fatty acids are predominant. There is controversy regarding both the degree to which these triglycerides are hydrolyzed before absorption and the extent to which the digestion products are absorbed in particulate or in water-miscible form. These two problems, one chemical and the other physical, are interrelated. Thus, so far as is known, the triglycerides, diglycerides and monoglycerides exist in the watery intestinal contents mainly in particulate form, either as unstable droplets and blobs or as a stable emulsion. The fatty acids, on the other hand, may be present either in particles or in water-miscible form, as soaps or as complexes with bile acids, depending on conditions in the lumen. Partial hydrolysis, then, would leave glycerides which are likely to be absorbed as particles. Whether hydrolysis is partial or complete, however, fatty acids are liberated, and these may be absorbed either as particles or in water-miscible form.

There is general agreement that a mixture of triglycerides, diglycerides and monoglycerides and free fatty acids is found in the lumen at all levels of the small intestine until absorption is complete (Blankenhorn and Ahrens, 1955; Ahrens and Borgström, 1956). However, this does not exclude the possibility that triglyceride is finally absorbed only as glycerol and fatty acids. An equilibrium may exist so that glycerides in the lumen are progressively hydrolyzed as fatty acid is absorbed into the epithelium. It would be valuable supporting evidence for incomplete hydrolysis if it could be conclusively shown that pancreatic lipase(s) did not completely hydrolyze long-chain triglycerides. *In vitro*, it seems that under suitable conditions triglyceride can be completely hydrolyzed (Borgström, 1952*b*). During the time spent by fat in the intestinal lumen, however, digestion may still remain incomplete, since pancreatic lipase splits the fatty-acid "ribs" from the glycerol "backbone" much more readily at the two ends of the molecule, positions 1 and 3, than in the middle, position 2. The problem is a complicated one, and was critically reviewed by Bergström and Borgström (1955).

The strongest evidence for incomplete hydrolysis and, as a possible corollary, for particulate absorption has come from experiments in which the glycerol and fatty-acid components of triglyceride are separately labelled. These experiments depend upon the well-attested fact that very little glycerol which has been split from long-chain triglyceride molecules by digestion combines with free fatty acid to form glycerides again, either in the

intestinal lumen or during passage through the epithelial cell (Bernhard *et alii*, 1952). Therefore, if a substantial proportion of labelled glycerol is found in glycerides in the epithelium or in the intestinal lymph, which carries away most of the absorbed long-chain fat (see later), it is fairly safe to assume that hydrolysis of the digestion mixture entering the epithelium has not been complete. The exact composition of the partially hydrolyzed material entering the cell will still be uncertain, however, because recombination of fatty acid with partially hydrolyzed glyceride can occur in the intestinal lumen (Borgström, 1954) and because further hydrolysis may occur in the epithelial cell. There is not space to review in detail the experiments of this type which have been done. The absorbed mixture has been studied in the intestinal wall (Skipski *et alii*, 1959) and in the lymph in rats (Reiser *et alii*, 1952; Reiser and Dieckert, 1956) and in man (Blomstrand *et alii*, 1959). The results of all these experiments are in general agreement that rather less than half of the fed triglyceride molecules are completely split to glycerol and fatty acids. The remainder give a mixture containing some triglyceride, but mainly diglyceride and monoglyceride and the fatty acids split off during partial hydrolysis. This view was further supported in man by experiments in which samples of intestinal contents were analysed after feeding triglycerides containing a proportion of labelled branched-chain fatty acids, which cannot readily be split off by pancreatic lipase (Borgström *et alii*, 1957).

It will be noted that the evidence supporting partial hydrolysis of some triglyceride molecules also shows that considerably more than half of the fatty acid originally combined in triglyceride will be set free. The physical state of this unesterified fatty acid as it enters the epithelial cell is difficult to assess. To form the smallest possible water-soluble complex, the molar ratio of bile salts to fatty acids would need to be 4 : 1 (Verzar and McDougall, 1936), and in man this is not achieved after a fatty meal (Borgström *et alii*, 1957). Moreover, the smallest complex would have a high molecular weight (2000 to 4000—Schmidt-Nielsen, 1946), and it is difficult to see how it could penetrate a cell membrane. Hydrotropes, such as bile salts, allow the formation of appreciable amounts of soap at pH 6·0 to 7·0, and there may be a continuous transfer of fatty acid from emulsion to bile-acid complex to soap, with final absorption as soap (Schmidt-Nielsen, 1946; and brief review in Davson, 1959). The other possibility is that fatty acid may be absorbed as particles. Singer *et alii* (1954) found that oleic acid became emulsified in an intestinal segment, a Thiry fistula, from which bile and pancreatic juice were excluded. The emulsified acid was readily absorbed, as shown by chylomicron counts in the blood-stream.

How soaps or bile-acid complexes finally penetrate the cell membrane is still obscure, but some issues connected with particulate absorption may be briefly considered. There is substantial evidence that fine emulsification is necessary. For details, one of Fraser's reviews (1958, 1960) should be consulted, but some of the main points are as follows.

A fine emulsion is normally seen in man and animals after a fatty meal, most of the particles being 0·5μ in diameter or less. A mixture of bile salts, fatty acids and monoglycerides in proportions comparable with those found during fat digestion forms a fine emulsion, which is stable *in vitro* over the range of pH 6·0 to 8·0, found in the intestinal lumen. In man, when bile acids or pancreatic lipase are absent, intestinal emulsification is defective and an ordinary fat meal is poorly absorbed, but absorption is rapid if finely emulsified fat is given. In animals, the chemically inert paraffins are not absorbed unless given either as a fine, stable emulsion or with digestible fat to provide a natural emulsion.

When selected evidence is put together in this way, the story is a convincing one, but there is still much information to be gathered. Quantitative data on size distribution and chemical composition of the emulsified droplets and on the amounts and types of fat in the aqueous phase might be instructive, though technically difficult to obtain. For example, Borgström (1960), after breaking the intestinal emulsion by high-speed centrifugation, found that about half the total lipids, including about half the diglyceride and monoglyceride, were present in the clear subnatant. This challenges the assumption that partial glycerides are not significantly water-miscible in the intestinal lumen. Further qualitative description is also necessary, especially in man, in whom the evidence is mainly restricted to assertions that a fine, stable emulsion was or was not present. Blankenhorn and Ahrens' (1955) intubation technique now makes it possible to determine the state of the emulsion at various levels of the small intestine and at various times after a meal, both in normal patients and in those with bile or pancreatic deficiency before and after replacement therapy. Comment on the appearance of the intestinal contents after ingestion of the fine emulsions which increased

chylomicron counts in bile or pancreatic deficiency would also be valuable.

On the assumption that a fine emulsion is important for absorption, there is still the problem of passage through the cell membrane. If the emulsion was disrupted on the cell surface, then fat might penetrate molecule by molecule. Such a change of state at the cell membrane might explain the interesting observations of Paul and Pover (1960). When an inert, oil-soluble silicone was mixed with olive oil and fed to rats, they found that the fat, but not the silicone, was absorbed, although a "normal" intestinal emulsion was formed. Rigid plastic particles, 0·01 to 1·2μ in diameter, are not absorbed, whether coated with hydrophilic or lipophilic material (Juhlin, 1959).

If the emulsion is not broken at the cell surface, then the particles with their hydrophilic coating may enter through preformed canals, or be pushed bodily through, or be carried through by pinocytosis, involving flow of the cell membrane. The last-mentioned possibility is supported by beautiful electron microscope observations in rats by Palay and Karlin (1959*a*, 1959*b*), whose papers should be studied in the original. Their results suggested that fat entered between the bases of the microvilli by pinocytosis of very fine particles, not greater than 65 mμ in diameter. It will be noted that these particles were much smaller than the limiting size for effective absorption which was found in Frazer's experiments. The particles were then carried through the cell in the complicated system of membrane-lined vesicles and channels which make up the endoplasmic reticulum. Thus they were inside the cell, but outside the cytoplasm proper. Finally, they were discharged—without a membranous coat—from the sides of the cell at about the level of the nucleus. As Palay and Karlin stressed, the quantitative importance of particulate absorption cannot be settled by electron microscopy. Indeed, it is difficult to see at present how resynthesis and rearrangement of triglyceride molecules, to the extent to which they are believed to occur during transit through the cell, can take place at the surface of the droplets.

Short-Chain Fatty Acids

The common dietary fats consist of mixed triglycerides, predominantly of long-chain fatty acids. Even in fats such as coconut oil, in which 70% of fatty acids are 12C, the glycerides will be virtually water-insoluble. So far as the individual free fatty acids are concerned, it is fairly generally accepted that their state during absorption and their fate thereafter are related to their partition between oil and water phases. This aspect is most conveniently considered in relation to pathways for absorbed material.

Absorption between Cells

The foregoing discussion implies that absorption takes place through epithelial cells and not between them. This view is supported by the evidence that fat absorption requires metabolic energy, has a limited capacity and involves considerable molecular changes. Also, electron microscopy suggests that adjacent cells are firmly held together near the free border, in the region of the terminal bars. Deeper, the lateral surfaces are plicated and less closely apposed. As Palay and Karlin (1959*b*) showed, particulate fat may escape from the cells here and come to lie between cells, but deep to the terminal bar. In light microscopy, with less critical resolution, this could easily give the impression that some particles passed in a continuous stream between cells.

CHANGES IN THE EPITHELIAL CELL

Long-Chain Fats

Hydrolysis and Resynthesis.—Since long-chain fat enters the epithelial cell partly or completely hydrolyzed, but leaves it almost entirely as triglyceride (Bergström and Borgström, 1955), it is clear that resynthesis occurs in the cell. The glycerol used for complete synthesis from glycerol and fatty acids is produced mainly in the cell. The source is not definitely known, but experiments with glucose-1-C_{14} suggest that this may act as a glyceride-glycerol precursor (Dawson and Isselbacher, 1960). It is generally considered that rebuilding of triglyceride from glycerol is not simply a reversal of the stages of digestive breakdown. Energy sources, including oxidative phosphorylation, are probably involved (Kennedy, 1957). Conjugated bile salts may have an intracellular function in promoting esterification of fatty acids with glycerol. This was demonstrated by Dawson and Isselbacher (1960) by incubation experiments with everted sacs of small intestine from a number of species, including man. While synthesis from glycerol may offer special biochemical problems, recoupling of fatty acids to absorbed monoglyceride and diglyceride may occur by re-esterification or transesterification, catalyzed by cellular fat-splitting enzymes. Such exchanges have been demonstrated in the intestinal lumen during digestion with pancreatic lipase (Borgström, 1954; Ahrens and Borgström, 1956).

Although finally resynthesized almost entirely to triglyceride, the digestion products may first

undergo further hydrolysis within the cell. This was demonstrated by Blomstrand *et alii* (1956), using triglyceride in which was incorporated a branched-chain fatty acid which formed ester linkages resistant to pancreatic lipase but not to epithelial esterases. However, the extent to which further hydrolysis of freely digestible fat takes place in the epithelial cell cannot yet be determined. Comparison of isotope labelling in luminal contents and lymph suggests that little glycerol is split off in the cell from absorbed glyceride, but that considerable rearrangement of the fatty-acid moiety takes place (Blomstrand *et alii*, 1959).

Phospholipid Turnover.—Although the phospholipid content of the intestinal wall does not increase appreciably after a fatty meal, there is evidence that these lipids are involved in fat absorption (Bergström and Borgström, 1955). Experiments in which choline, glycerophosphate or lecithin was added to a fatty meal (Frazer, 1958) are difficult to interpret. In the cell, however, some absorbed fatty acid is undoubtedly incorporated into phospholipid. The extent to which this occurs varies with the type of fatty acid; saturated acids are incorporated much more readily than unsaturated acids (Hanahan and Blomstrand, 1956) and incorporation of saturated acids is greater with longer chain lengths (Borgström, 1952*a*). Whether whole phospholipid molecules are broken down and resynthesized more rapidly during fat absorption is difficult to determine. Many workers have found an increased incorporation of radioactive phosphate, but Zilversmit *et alii* (1948) did not. In any case, only a small proportion of absorbed fatty acid reaches the lymph as phospholipid (Bloom *et alii*, 1951; Blomstrand and Dahlbäck, 1960). Thus phospholipid does not seem to be quantitatively important in carrying absorbed fat away from the epithelium, though it may be an important component of the emulsifying system for chylomicrons (Elkes and Frazer, 1943). This group of lipids may also be important in other ways—for example, in the movement of absorbed fat through the epithelial cell; or the increased turnover may be merely a consequence of the increased intracellular pool of fatty acids and other products during fat absorption.

Structural Changes in Absorbed Fatty Acids.—Comparison by efficient fractionation techniques of the composition of triglycerides in lymph with those administered suggests that long-chain fatty acids are not substantially modified during absorption—for example, by hydrogenation or dehydrogenation or by shortening or lengthening of the carbon chain (Bragdon and Karmen, 1960; Blomstrand and Dahlbäck, 1960). Very little, if any, absorbed long-chain fatty acid is "lost" by oxidation during passage through the epithelial cell, as shown by the high recoveries of isotopic label in lymph triglycerides and by the small proportion of absorbed label which is expired as $C^{14}O_2$ in the presence of a lymph fistula (Blomstrand, 1954). Fatty acids with a shorter chain length—for example, decanoic (10C)—are metabolized more rapidly (Blomstrand, 1955); but it is difficult to determine how much of the expired $C^{14}O_2$ is produced in the intestinal epithelium. Only a small proportion of such acids passes into the lymph, and therefore establishment of a lymph fistula does not preclude oxidation elsewhere.

Short-Chain Fatty Acids

The substantial differences between the handling of these and of long-chain fatty acids in the mucosal epithelium are more conveniently dealt with under pathways for removal of absorbed fat.

Removal of Absorbed Fat

Much argument and experimental effort has been devoted to the factors which determine how much of the absorbed fat is carried away in the intestinal lymph and how much by the portal blood-stream. During the last 12 to 15 years, progress has been accelerated by the use of isotopes, by techniques for deliberate collection of lymph from unanæsthetized animals and even from man, and by improvements in methods of separating and identifying fatty acids and lower glycerides. For a full, critical discussion, the review of Bergström and Borgström (1955) should be consulted.

Factors Determining the Pathway

Length of Fatty-Acid Chain.—Short-chain, low molecular weight fatty acids do not pass into the lymph. Thus, feeding tributyrin (4C) mixed with other fats did not significantly affect the volatile fatty-acid concentration in the thoracic duct lymph of anæsthetized dogs (Hughes and Wimmer, 1935); nor did 0·5 ml. of tributyrin increase the output of esterified fatty acid during a period of six hours in unanæsthetized rats (Simmonds, 1955*b*).

When isotopically labelled fatty acids were fed, some label appeared in the lymph after 10C acids, with increasing recoveries up to 14C (Bloom *et alii*, 1951; Borgström and Tryding, 1956). With long-chain fatty acids ($\geqslant$16C), results are consistent with passage of nearly all the fatty acid into lymph. In these experiments, the pure fatty acids or triglycerides were

given in tracer doses, dissolved in vegetable oil. Evidence for the same differences in pathway has also been found when dietary fats were fed in which the mixed triglycerides contained an unusual proportion of medium-chain or short-chain fatty acids. Experiments which involve the handling by the intestinal epithelium of considerable amounts of fatty acids of different chain lengths are important, because they may involve factors, such as solvent capacity of fluid or other constituents of cells, which are not crucial when tracer doses are fed. Thus, little or no fatty acids below 10C were found in the lymph in a child with chylothorax (Fernandes *et alii*, 1955) or in a patient with chyluria (Blomstrand *et alii*, 1958). After feeding coconut oil, rich in 12C fatty acids, the concentration of 12C acids in chylomicron triglycerides was much lower in the lymph of rats and in the serum of man than in the dietary fat. After olive oil, which consists mainly of long-chain fatty acids, the chylomicrons contained saturated and unsaturated 16 and 18C acids in almost the same proportions for each category as in the olive oil (Bragdon and Karmen, 1960). When unanæsthetized rats were absorbing coconut oil (rich in medium-chain fats) at a steady rate, fat disappeared from the intestinal lumen at a rate considerably greater than the steady output of esterified fatty acid in the lymph (Bennett and Simmonds, to be published; Shepherd and Simmonds, 1959).

Degree of Hydrolysis in the Intestinal Lumen.—It is now generally accepted that long-chain fats, whether absorbed into the cell as fatty acids or as glycerides, leave the cell mainly as triglycerides and pass almost entirely into the lymph. In earlier formulations of the partition hypothesis (Frazer, 1946), it was held that long-chain fatty acid after absorption passed into the portal vein, while neutral fat passed into lymphatics. The evidence is heavily against such a differentiation (Bergström and Borgström, 1955), and it is no longer regarded as a basic tenet of the partition hypothesis (Frazer, 1955). However, qualitative observations after feeding dyed oleic acid and dyed olive oil, respectively, to rats (Frazer, 1943) are still quoted in textbooks. It may, therefore, be worth mentioning that repetition of the dye experiments gave no quantitative differences between oleic acid and olive oil in the lymphatic recovery either of dye or of esterified fat (Simmonds, 1955*a*).

Ease of Re-esterification.—Although the degree of hydrolysis of fat entering the epithelium does not influence the pathway of removal, evidence suggests that the degree to which the fat is re-esterified by the time it leaves the epithelium is an important factor. This is understandable, since the glycerides, chiefly triglycerides, in lymph are found almost exclusively in chylomicrons (Hillyard *et alii*, 1958), and since the lymphatics are the only effective pathway for removal of particles from tissue fluids (Yoffey and Courtice, 1956).

The clearest evidence for esterification as a factor promoting lymphatic removal has been given by the use of non-dietary lipides—isotopically labelled branched-chain fatty acids, dimethylstearic and dimethyl nonadecanoic acid, and cholanic acid. All three substances resemble the long-chain dietary fatty acids in solubility. However, only a very small proportion of cholanic acid—which is not esterifiable—passes into the lymph after absorption (Sjovall and Akesson, 1955). The branched-chain fatty acids are esterified in the mucosa, although to a lesser extent than long-chain fatty acids, and are removed partly, but not exclusively, by lymphatics (Borgström and Tryding, 1956; Blomstrand *et alii*, 1956).

So far as dietary fats are concerned, it has been suggested (Bloom *et alii*, 1951) that the increase in lymphatic removal of medium-chain fatty acids (C_{10}–C_{14}) with increasing chain length reflects increasing incorporation in chylomicron triglycerides. It has been shown for isotopic decanoic acid (C_{10}) that most of the label in lymph is in triglyceride (Blomstrand, 1955; Borgström and Tryding, 1956), while most of the label in portal venous blood is in the free fatty-acid fraction (Borgström, 1955).

Oil-Water Partition.—As the above evidence shows, the removal of absorbed lipid by lymph or by portal blood-stream is not determined simply by the degree to which it enters lipid or aqueous phases respectively *in vitro*. Indeed, the partition hypothesis (Frazer, 1955) cannot readily be put to a quantitative test. Too little is known of the solvent properties of lipids in cell membrane and cytoplasm, on the one hand, and, on the other, of substances in cell and tissue fluid which might bring otherwise insoluble lipid into a state of fine dispersion in aqueous fluid. Also, very little is known of factors which determine the ease with which finely dispersed lipids penetrate the walls of blood capillaries. For example, unesterified fatty acids readily form a water-soluble complex with albumin, but exchange across the capillary wall much more rapidly than albumin (French *et alii*, 1958). This is a crucial point, since our knowledge of lymphatic absorption in many regions (Yoffey and Courtice, 1956) suggests that lymphatic removal of material becomes

important chiefly when penetration of the blood capillary wall becomes very difficult. Blood flow is so much greater than lymph flow that a substance to which the capillary wall is only moderately permeable may still be removed mainly by the blood-stream, despite the high permeability of lymphatics. On this basis, removal of butyric acid by the portal blood and of particulate mixed triglyceride as chylomicrons by lymphatics is readily understandable. But more knowledge of capillary permeability and of the physical and chemical changes in lipids during absorption is needed before the removal of medium-chain fatty acids is fully understood, or before an explanation can be offered of fat absorption without chylomicronæmia, as in the case described by Salt *et alii* (1960), or of the alteration in pathway in biliary deficiency which was suggested by Borgström's (1953) results.

Lymph Flow

During absorption of moderately large amounts of fat, there is a considerable increase in intestinal lymph flow. The mechanism of this well-known effect is not understood. It does not appear to be due merely to the concurrent absorption of large volumes of digestive fluids (Borgström and Laurell, 1953). It does not occur with fat-free food or with meals of pure carbohydrate or protein (Simmonds, 1954, 1955*b*). It seems to be due to an increase in capillary filtration of a rather unusual kind. The clinical significance of the phenomenon is hard to assess, but the pharmacology of the substance or substances which cause a two-fold to three-fold increase in lymph flow in unanæsthetized rats may be of some interest; 5-HT has not been implicated (Simmonds and Toh, 1957).

Absorptive Capacity

Much of the work on the absorptive capacity of the small intestine for fat and on factors affecting it (Deuel, 1955) has not taken into account variations in the rate of stomach emptying, which would limit access to the absorptive area in the small intestine. Tidwell (1958) has reemphasized this point, and it has been shown in rats that the maximum absorption rate was determined by the maximum rate at which fat left the stomach (Aberdeen *et alii*, 1960). However, with steady intraduodenal infusion of emulsified fat it has been shown that the rate at which fat can be steadily transferred to the lymph is limited (Shepherd and Simmonds, 1959). The same rate of fat infusion gave maximal values for disappearance of fat from the lumen (Bennett and Simmonds, to be published). Such experiments indicate clearly that there is a limit to the rate at which fat can be absorbed through the epithelium. The steady-state technique, although time-consuming, promises to be useful in defining factors which affect disappearance of fat from the lumen or its subsequent transfer to lymph.

At the moment it is not possible to say which of the many processes involved are limiting factors in normal fat absorption. In the present article, attention has been concentrated on the nature of these processes, so far as triglyceride fat is concerned, rather than on factors which may modify their operation. Evidence has been drawn chiefly from experiments on laboratory animals, so that the reader may consider for himself the findings in relation to clinical studies, including those in the malabsorption syndrome. However, reference has been made to important advances in knowledge of fat absorption in normal man, with new clinical techniques and refined chemical procedures. Doubtless these will be applied increasingly in pathological conditions. Finally, experiments such as those of Borgström *et alii* (1957) on the absorption of a mixed meal in man are of great importance. For technical convenience, much experimental work has been done on rats which have been given a single fairly large dose of fat alone, after a period of fasting. While this needs no justification for particular experimental purposes, the results need to be compared with those under more usual dietary conditions before general conclusions are drawn.

SUMMARY

A brief review of triglyceride absorption is given, with particular reference to papers published during the last five years. Absorption of a substantial amount of incompletely hydrolyzed fat is well documented, but the proportion of fed triglyceride which is so absorbed and the composition of the absorbed mixture are uncertain. Physically, some fat seems to be carried into the cell as very finely emulsified particles by pinocytosis or membrane flow, but the quantity so handled has not yet been determined. The way in which the rest of the fat, including water-miscible complexes, passes through the cell membrane is unknown. Inside the cell, complex molecular changes take place, including resynthesis of triglyceride and exchanges with phospholipid. Physically, a stream of droplets can be identified in the endoplasmic reticulum, but the extent to which these participate in biochemical exchanges is unknown. Fat eventually leaves the cell in one of several states. Whether they enter the

cell as fatty acids or as lower glycerides, the triglycerides of long-chain fatty acids emerge from the cell almost exclusively as triglycerides in the form of emulsified particles, chylomicrons, which are removed almost entirely in lymph. Any very short-chain fatty acids, such as butyric acid, are split off and removed in solution by the blood-stream. The same is true of much of the 10C fatty acids, of some of the 12C acids and of a small amount of longer-chain fatty acids. The factors involved in this partition require further investigation.

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Proceedings of The Royal Australasian College of Physicians

ANNUAL MEETING, 1961

The Annual Meeting of the College in 1961 was held in Perth from May 1 to 3. It was attended by 107 Fellows and Members representative of all the Australian States and New Zealand. The President, Dr. T. M. Greenaway, was in the chair. Overseas guests of the College at this meeting were: Dr. Eugene Braunwald, R. T. Hall Trust Lecturer for 1961 Professor E. G. L. Bywaters, Professor of Rheumatology at the Postgraduate Medical School, London; and Professor C. C. de Silva, Professor of Pædiatrics at Colombo University.

COLLEGE CEREMONY

The Annual Ceremony of the College was held in the Winthrop Hall of The University of Western Australia on Tuesday, May 2, in the presence of His Excellency the Lieutenant-Governor of Western Australia, Sir John Dwyer, K.C.M.G. An audience of 650 was present. Addresses were given by the President and His Excellency the Lieutenant-Governor.

Newly admitted Fellows and Members were presented to the President. The Honourable Frank S. Wise, M.L.C., then delivered the Arthur E. Mills Memorial Oration entitled "Australia's Empty Spaces". At the conclusion of the Ceremony guests were entertained at supper in the Refectory of the Guild of Undergraduates.

COLLEGE DINNER

The College Dinner was held at the Esplanade Hotel, Perth, on the evening of Wednesday, May 2. Guests at this function were Dr. Eugene Braunwald, Professor E. G. L. Bywaters, Professor C. C. de Silva, Professor Gordon King (representing the Chairman of the Australian Regional Council of the Royal College of Obstetricians and Gynæcologists), Mr. Hector Stewart (representing the President of the Royal Australasian College of Surgeons), Sir William Morrow (President of the Gastro-Enterological Society of Australia), the Honourable Frank S. Wise (Arthur E. Mills Orator) and Dr. Ronald Winton (Editor of *The Medical Journal of Australia*). The toast of the College was proposed by Dr. H. Maynard Rennie and acknowledged by the President. The toast of the guests was proposed by Dr. Bruce Hunt and replied to by Mr. Hector Stewart.

SCIENTIFIC AND CLINICAL PROGRAMME

Two scientific sessions, a plenary meeting with the Gastro-Enterological Society of Australia and two clinical meetings were held in the Main Lecture Theatre of the Royal Perth Hospital.

First Scientific Session

At the first scientific session on Monday, May 1, the following contributions were presented.

In a paper by R. A. Joske and B. F. Vaughan entitled "The Radiological Diagnosis of Atrophic Gastritis and Gastric Atrophy", Dr. Joske described a radiological picture termed radiological gastric atrophy (R.G.A.). This consisted of the presence of a long tubular hypotonic stomach, with absent rugal markings on the fundus and greater curvature when the stomach was distended, or in other cases "tissue paper" gastric folds. The relation between this radiological picture and mucosal histology was discussed in a series of 160 patients. If gastric biopsy gave normal results, R.G.A. was not present in any case; if biopsy showed gastric atrophy, R.G.A. was present in all cases. Between those extremes, increasing mucosal abnormality was reflected in an increasing frequency of R.G.A. Those relations did not hold in the presence of a gastro-duodenal lesion, such as peptic ulcer or partial gastrectomy. R.G.A. was found to be more frequent in the elderly and to be associated with abnormalities of vitamin B_{12} absorption.

A paper entitled "The Disposal of Intravenously Administered Isotopically Labelled Chylomicrons Studied in Normal Subjects, Patients with Ischæmic Heart Disease and Patients with Essential Hyperlipæmia", by Dr. P. J. Nestel and Dr. M. Denborough, was presented by Dr. Denborough, who was introduced by Dr. R. B. Lefroy. He reported that abnormally intense and prolonged lipæmia after fatty meals had been described in patients with ischæmic heart disease and in patients with essential hyperlipæmia. Alimentary lipæmia represented the difference between absorption into and removal from the blood-stream. That study had been concerned with two aspects of chylomicron removal. (*a*) The possibility that chylomicrons were removed more slowly from the blood of one group than from that of the other because of differences in the physical state of the chylomicrons had been tested in the following manner. Subjects from various groups were fed a fat meal containing C_{14}-labelled tripalmitin. They were bled three hours later, and chylomicron infusions were prepared from the plasma. Matched pairs of those infusions were then

infused into common recipients and the rates of disappearance of chylomicrons were measured. (*b*) The possibility that chylomicrons were removed more slowly because of abnormalities in the removal mechanism had been tested in the following way. A fat meal containing C_{14}-labelled tripalmitin was fed to a subject with a thoracic duct fistula, and standard labelled chylomicrons were prepared from the draining chyle. Those standard infusions were reinfused into various groups of subjects and the rates of disappearance of chylomicrons were measured. The results of those two procedures, which had been carried out on eight patients with ischæmic heart disease, in eight matched controls and in two patients with essential hyperlipæmia, were presented and discussed.

Professor E. G. L. Bywaters spoke on " The Vascular Lesions of Rheumatoid Arthritis and Other So-called Collagen Diseases ". He said that they were of three kinds: acute inflammatory lesions of small vessels, mainly in muscle; lesions similar to polyarteritis nodosa; and finally, a type of obliterative endarteritis first described by him in 1957. The last appeared to be the most frequent type of lesion, and might occur in many different sites—limbs, viscera, muscle, etc. It might be associated with gangrene or infarction, with neuritis or with ulceration, and was usually seen in patients with nodules and a positive response to the Rose Waaler test. Recent angiographic studies with colleagues at the Postgraduate School of Medicine (Dr. Steiner, Dr. Dixon, Dr. Laws, Dr. Scott, Dr. Hourihane and Dr. Doyle) had shown multiple blocks in the digital arteries of patients with rheumatoid arthritis due to such obliterative intimal thickening, even in patients without Raynaud's phenomenon and with a normal response to the reactive hyperæmia test; some had shown simultaneous arteritic lesions in the viscera. Although such lesions had occurred before the introduction of steroids, they seemed to be more common now, particularly in those more severely incapacitated patients treated with steroid. That might possibly be associated with the tendency in such patients for the Rose Waaler titre to rise with time, compared, for instance, with a series treated by gold. The therapeutic implications of the observation were discussed.

Dr. H. Lander presented a paper entitled " The Pattern of Erythrocyte Destruction in Hæmolytic Syndromes ", in which he briefly described the method of labelling red cells with chromium-51 and of performing red-cell survival and " organ uptake " studies by " surface counting ". He reported that 48 patients suffering from a variety of hæmolytic syndromes had been studied by that method. The pattern of erythrocyte destruction which had been found in those cases was presented. Surface counting was performed in all cases over the heart, liver and spleen. In certain cases, measurements were also performed over both lungs, the kidneys, the sacrum, a tibia and any large abnormal mass or masses. Four major patterns of radio-chromium uptake with respect to those organs had emerged: Type I, in which no significant " uptake " occurred; there was a steady fall-off in activity in all organs counted; Type II, in which " uptake " occurred only in the liver; Type III, in which " uptake " occurred in both the liver and spleen; Type IV, in which " uptake " occurred only in the spleen. In those cases in which only splenic " uptake " occurred (Type IV), three minor patterns were also evident: group (*a*), in which a rapidly progressive " uptake " occurred in the spleen; group (*b*), in which the initial splenic count exceeded the heart count, but then decreased at a rate equal to that of the heart count; group (*c*), in which there occurred only a slowly progressive uptake in the spleen. Evidence was also presented which suggested that under certain pathological conditions the bone-marrow and lungs might also become a major site of red-cell destruction.

Second Scientific Session

At the second scientific session held on Tuesday, May 2, the following contributions were presented.

In a paper entitled " Selective Angiocardiography in the Selection of Patients with Cardiac Surgery ", Dr. Eugene Braunwald said that a wide variety of special diagnostic methods were now available, by means of which the physician might evaluate patients with congenital or acquired heart disease. The information derived from the clinical examination and catheterization of the right and left sides of the heart would ordinarily indicate the presence and severity of valvular stenosis and the sites of origin and termination, as well as the magnitude, of circulatory shunts. However, the specific anatomical defect which was present could not ordinarily be determined with certainty from such studies. A precise anatomical diagnosis could be established preoperatively only with direct visualization by means of contrast radiography. With the widespread utilization of open-heart surgery, the physician must no longer be satisfied with merely classifying the general nature of the lesion, but must provide the surgeon with a clear definition of the anatomical features of the anomaly as well as of its physiological sequelæ; such precise knowledge was essential to intelligent surgical planning. In " selective " angiocardiography, a contrast substance was delivered into the central circulation through a cardiac catheter. In that technique, the opaque medium might be delivered in high concentration at the site deemed most likely to reveal best the anatomical abnormality present. When certain precautions were observed, the procedure was associated with little risk, and furnished diagnostic information which could not be obtained by any other means. Although visualization of the cardiac chambers and great vessels was usually possible after the intravenous injection of a contrast substance, the disadvantages of that method were becoming increasingly apparent. With intravenous injections, the medium was greatly diluted in its passage through the circulation, and the contrast obtained might be inadequate. The left side of the heart and aorta, in particular, were usually visualized only poorly after an intravenous injection. Furthermore, simultaneous opacification of adjacent structures might obscure the specific area under investigation. The technique of selective angiocardiography and aortography was described and its value was demonstrated by means of specific examples.

George V. Hall presented a paper entitled " The Relationship of Effort to the Onset of Myocardial Infarction: A Review of 500 Cases ", in which he said that 500 cases of myocardial infarction, electrocardiographically proved, had been reviewed. A special point made in the paper was the relationship of effort to the onset of myocardial infarction. Cases of acute coronary insufficiency without infarction had been excluded. It was found that 83% of myocardial infarcts had occurred whilst the patient was at rest. Of those, 20% had occurred whilst the patient was in bed at night, 4% had occurred whilst patients were in hospital for some other disease, and 1% had occurred after operation. In 7% the onset had coincided with heavy effort or unusual excitement. In 9% the infarcts had begun during gentle exercise, such as walking at a normal pace, or during work involving slight or moderate exertion to which the patient was accustomed. It was concluded that by far the largest

numbers of myocardial infarcts occurred whilst the patient was seated or walking quietly about his home, or when he was in bed at night. A much smaller number occurred during quiet exercise, and in an even smaller number the onset of infarct coincided with heavy exertion or unusual excitement. However, one could not agree with Master's conclusions based purely on statistical evidence that effort was never related to myocardial infarction. It was held that one could not ignore the coincidence of heavy effort or unusual excitement and myocardial infarction when it occurred. The possible mechanism of the relationship was discussed. Premonitory symptoms had preceded the onset of infarction in 38% of that series, and their significance was discussed.

"Local and Reflex Mechanisms in Fluid Aspiration" was the subject of a paper by H. J. H. Colebatch and D. F. J. Halmagyi, and presented by H. J. H. Colebatch. Dr. Colebatch said that, as a result of previous work, the hypothesis was proposed that the severe effects of small quantities of fluid in the airways was due to its relatively high surface tension. In the experiments reported, both local and reflex factors had been investigated. The experiments were performed on lightly anæsthetized, intubated sheep, and the fluid was injected into the trachea. One millilitre per kilogram of a low surface tension fluid had the same effect as fresh water, even when foaming was prevented. In vagotomized animals the response was reduced in relation to the intact animal, but again was similar to fresh water. After the exhibition of 0·2 mg. per kilogram of atropine, 1 ml. per kilogram of water caused a fall in lung compliance of only 26%, compared with a 50% fall in vagotomized animals and a 65% fall in intact animals found previously. In atropinized animals, neither the surface tension of the fluid nor the tendency to foaming appeared to affect the fall in lung compliance. Dr. Colebatch said the results indicated that the gross compliance fall produced by small quantities of water and the accompanying hypoxæmia were due to a reflex which resulted in airway closure. That reflex depended on nervous structures within the lung and was largely independent of the central nervous system. It was probably of major importance in determining the reaction of the lung, not only to fluid, but also to other external irritants. The implications of those findings with regard to the treatment of patients suffering from aspiration of fluid were discussed.

N. McK. Bennett presented a paper entitled "A Study of Drug-Induced Agranulocytosis and Septicæmia", in which he said that 10 patients with drug-induced agranulocytosis or neutropenia had been admitted to Fairfield Infectious Diseases Hospital, Victoria, during the last three years. Those cases illustrated the potentiality of thiouracil, phenothiazine and pyrazolidine compounds to cause bone-marrow depression, and the capacity of neutropenia to predispose to septicæmia of an unusual type. All the patients had been admitted with a severe febrile illness commonly associated with exudative tonsillitis and jaundice. Clinical diagnosis of septicæmia was confirmed bacteriologically in three cases. The causative organisms in those three cases were unusual, and indicated that initial chemotherapy should be broad enough to cover such infections. In otherwise healthy patients, treatment with penicillin, streptomycin, erythromycin and corticosteroids had been successful. The diagnosis, hæmatological findings, complications and treatment of the patients were described. The proposal, probably fallacious, that some types of septicæmia might initiate agranulocytosis was briefly discussed in relation to those cases.

A paper entitled "The Mentality and Personality of the Julio-Claudian Emperors" was presented by Gerald C. Moss. Dr. Moss said that it had been pointed out that it was difficult to draw a picture of Claudius at once satisfying to the historian, physician and psychologist, and that was true of all the Julio-Claudian emperors. The ancient writers tended to over-emphasize their personal characteristics, and views of character had continued to colour estimates of skill and ability and vice versa. Psychiatric diagnoses of the emperors had been made, and psychological speculation had often involved an attack on Tacitus' psychology. The evidence was overwhelming, even in the ancient histories, that Claudius' intellect was much better than had often been supposed. Organic and mental symptoms had often been confused, but archæology had done much to rehabilitate him. However, the acquittal of an emperor of a charge of mental disorder or vicious personality made it even more necessary to explain some of his actions, especially his severe measures against so many prominent citizens. It was not to be expected that genealogy would have any help to offer, but great importance could be attached to the restless, seething general environment. It was an age of cruelty. Conflict with the senatorial hierarchy and the birth of development of the oligarchic opposition had great significance. The law of *maiestas* was invoked on the vaguest pretext. The plight of the emperor had to be considered as well as that of his subject. His position was a peculiar one; if any prominent citizen was in fear of his life, so too was he. The effect on him of repeated dire suggestion could well have brought about his collapse; and it was submitted that in Rome the emperors had been subjected to suggestion of a nerve-racking form. The parallel of the indoctrination of prisoners in the hands of Communist captors, to take only one example, came to mind. As Sargant had pointed out, it was normal people who were sensitive to, and influenced by, what was going on around them, and it was the lunatic who was impervious to suggestion. Dr. Moss said that it could not, of course, be shown that the emperors were normal, normality or abnormality being extremely difficult to define. When it was remembered that an age was being considered in which the cultural and religious beliefs were utterly different from those of the present day, the difficulty was an insuperable one. The application of Pavlovian principles, as they had been applied in psychiatry, to a study of the emperors' personalities would be interesting, even if it involved the psychological speculation which had, often with good reason, been deplored. To shift the emphasis from where Tacitus laid it in his explanations of human behaviour, and to restate the interdependence, so well understood by Thucydides, of history and human nature was, it was hoped, not necessarily over-speculative or destructive to Tacitus' psychology.

Plenary Session

At the plenary session arranged by the Gastroenterological Society of Australia on Wednesday, May 3, the following contributions were presented.

A paper entitled "Patterns of Vitamin B_{12} Absorption", by W. R. Pitney, M. F. Carruthers and J. B. Stokes, was presented. Dr. Pitney reported that the absorption of orally administered radioactive vitamin B_{12} had been studied with a modified urinary excretion test. After an oral dose of 0·6 μg., 98 normal subjects had excreted from 14·2% to 39·6% of administered radioactivity (mean 25·0%, S.D. 5·9%). Eighty-nine patients with pernicious anæmia had excreted from 0 to 10·9% (mean 2·9%). When these patients were

given the dose of radioactive vitamin B_{12} with a preparation of hog intrinsic factor, excretion had ranged from 10·6% to 42·0% (mean 22·5%). The excretion pattern was similar whether the patient was in relapse or in remission after therapy. Nineteen of 20 patients with intestinal malabsorption (excluding that secondary to gastric surgery) had been unable to absorb radioactive vitamin B_{12} normally. When the dose was given with intrinsic factor in those 19, one of three patterns of absorption was observed: in one patient absorption became clearly normal, in some absorption was improved but still subnormal, while in the remainder absorption remained the same or actually decreased. The last-mentioned was the typical malabsorption pattern. There was overlap between patterns seen in pernicious anæmia and in intestinal malabsorption, and the interpretation of the results obtained in an individual patient might be difficult. The pattern was further confused by the existence of pernicious anæmia patients refractory to hog intrinsic factor. Multiple testing with both hog and human intrinsic factor might be necessary to determine the mechanism underlying inability to absorb radioactive vitamin B_{12}. After partial gastrectomy, failure to absorb vitamin B_{12} might be due either to loss of endogenous intrinsic factor secretion or to intestinal malabsorption.

D. J. Fone presented a paper entitled " Diverticulosis of Small Intestine ", in which he said that diverticula occurred in the small intestine (excluding the duodenum) in 0·1% to 1·0% of individuals, being more common over the age of 50 years. They were pseudo-diverticula, were situated along the mesenteric border and varied in their size and number. Though many were " silent ", abdominal symptoms might occur from stagnation and infection of fæcal content, intestinal obstruction, perforation or hæmorrhage. In the last six years the association of megaloblastic anæmia, steatorrhœa and other defects of intestinal absorption had been recognized. Thirteen cases were presented, which had been studied with Dr. W. T. Cooke in Birmingham, England. Of those patients, 10 had had symptoms due to the diverticula, consisting of recurrent abdominal pain, subacute bowel obstruction and recurrent diarrhœa. Five of the patients had had steatorrhœa with a daily fæcal fat excretion of 8 to 35 grammes, and macrocytic anæmia; the bone marrow was megaloblastic and the serum vitamin B_{12} concentration was below normal in all four examined. The absorption of radioactive vitamin B_{12} had been impaired in two of the patients, but had improved after treatment with achromycin. In a third there had been a severe intrinsic factor deficiency, that patient also having achlorhydria and gastric atrophy. Although the macrocytic anæmia was usually due to vitamin B_{12} deficiency resulting from bacterial competition, there might occasionally be an intrinsic factor deficiency and gastric atrophy similar to that of pernicious anæmia; gastric biopsy and vitamin B_{12} absorption studies might be necessary to distinguish the two mechanisms. Intermittent administration of broad-spectrum antibiotics often gave good symptomatic relief. Resection of the diverticula might occasionally be possible and effective, although recurrence might take place at a later date.

A paper entitled " Progressive Hepatitis: A Study of 25 Patients ", by C. R. B. Blackburn, J. Rankin, R. Beal and Mrs. A. Gannon was presented by Professor Blackburn, who said that progressive hepatitis had been defined as a diffuse inflammatory disease of the liver which progressed to cirrhosis, especially to multiple nodular hyperplasia, and which was of uncertain cause. The disease was differentiated from continuous or persisting hepatitis which did not progress to cirrhosis. The patients had represented all states of the disease, from acute inflammation to inactive cirrhosis. It had not been possible to exclude acute infectious hepatitis or serum hepatitis as ætiological agents, but none of the patients described had had a history of alcoholic excess, had been known to have been exposed to toxic drugs or chemicals or had had brucellosis, tuberculosis or sarcoidosis, and none had had recognizable acute diffuse lupus erythematosus, periarteritis or diffuse angeitis. It was suggested that progressive hepatitis represented an hepatic reaction to a variety of causes, and it was recognized that there was evidence for the participation of an immune type of reaction. The presence of non-hepatic features was emphasized, in particular the incidence in women, the presence of arthralgia, colitis and chronic diarrhœa, and renal changes. Two patients had had the nephrotic syndrome, and three others had had demonstrable renal changes. Studies of hormone excretion were being carried out by the use of chromatographic techniques, and a substance, moving at the same rate as pregnanetriolone, had been found to occur in most patients with progressive hepatitis, but in few with cirrhosis of alcoholic origin. The substance had not yet been identified. The chronic course of the disease was emphasized—that five of the seven males had died within seven years, but only five of the 18 females in two to 17 years. Causes of death had usually been liver failure and bleeding, but renal failure and carcinoma of the liver had each occurred once. Four patients died within five years of the onset, but 14 lived or were living more than five years from the beginning of the disease. Management consisted of bed rest and the administration of steroids. The difficulty in determining the value of either rest or steroids was emphasized, and it was pointed out that present practice was to administer steroids so long as the serum transaminase levels were significantly elevated.

In a paper entitled " Gastro-Intestinal Manifestations of the Collagen Diseases ", Sir William Morrow reported that 135 patients admitted to the Royal Prince Alfred Hospital during the ten-year period 1950–1959 in whom the diagnosis of systemic lupus, polyarteritis nodosa, scleroderma or dermatomyositis was acceptable, had been reviewed with particular reference to manifestations of gastro-intestinal disease. There had been 40 patients suffering from systemic lupus, 20 of whom had had evidence of gastro-intestinal disease. Of 45 patients suffering from polyarteritis, 10 only had had alimentary signs or symptoms. Systemic sclerosis or scleroderma had been noted in 39 patients, 31 of whom had had evidence of gastro-intestinal disease, as had six out of 11 patients with dermatomyositis. Of the total of 135, 67 patients (50%) had had evidence of alimentary disease, and 17 had presented with such signs or symptoms, which at times had remained the only evidence of polysystemic disease for weeks or months. The diagnosis of scleroderma or systemic sclerosis had seemed unacceptable in the absence of some gastro-intestinal abnormality.

Dr. Stanley Goulston, in a paper entitled " Painless Chronic Pancreatitis ", stated that pain was the outstanding symptom of pancreatitis, acute, subacute and chronic relapsing. That pancreatitis could exist without pain had been known for over 50 years; it had been described by Freidrich in 1878 and Opie in 1906. Nevertheless, apart from Bartholomew and Comfort's classic paper in 1956, few well-documented case reports of painless chronic pancreatitis had been reported. Four patients with chronic pancreatitis had been admitted to Royal Prince Alfred Hospital,

Sydney, during the period 1959–1960. A retrospective study of autopsy material had led to the discovery of six additional cases. The four patients had been males in the sixth and seventh decades. They had presented with steatorrhœa, weight loss and diabetes. The diagnosis had been established by eliminating other causes of steatorrhœa, by normal findings on jejunal biopsy, and in three cases by laparotomy, required to rule out pancreatic carcinoma. Histopathological examination of pancreatic biopsy specimens had demonstrated a distinctive pathology absence of exocrine tissue and its replacement by lymphoid accumulation with maintenance of lobular architecture. Both perilobular and intralobular fibrosis had been present. Islet tissue had tended to be preserved. Ducts had been present, and had not been dilated or involved in any inflammatory process. The ætiology was unexplained. None of the usual causes of chronic pancreatitis had been present. The two most attractive hypotheses were an autoimmune mechanism or pancreatic vascular disease. Evidence in favour of the latter was presented. These patients had responded favourably to low-fat, high-protein, average-carbohydrate diet, pancreatic and vitamin supplements and diabetic control. The prognosis appeared to be good, death occurring usually from vascular causes such as arterial thrombosis, hypertension and cardiac failure. Importance of the recognition of painless chronic pancreatitis lay in its response to therapy and in the measure of reassurance that could be given to the patient when the diagnosis was established.

CLINICAL MEETINGS

Two clinical meetings were held in the Main Lecture Theatre of the Royal Perth Hospital on Monday, May 1, and Wednesday, May 3. At the first clinical meeting the following contributions were presented: "Primary Hyperparathyroidism" by Professor E. G. Saint; "Lymphoblastic Lymphosarcoma", by J. Calder; "Renal Acidosis with Acquired Hæmolytic Anæmia and Hæmatemesis", by W. R. Pitney; and "Renal Tubular Acidosis with Purpura Hyperglobulinæmia (Walderstrom)", by A. K. Cohen.

At the second clinical meeting, the following contributions were presented: "Diabetes Mellitus with Remission During Chlorpropamide Treatment", by B. A. Hunt; "Infectious Hepatitis with Viral Encephalitis", by J. B. Stokes; "Congenital Cerebral Vascular Malformation", by A. Fisher; and "Autism", by Professor W. B. Macdonald.

OFFICE-BEARERS

The constitution of Council for the period 1961–1962 is as follows:

President: T. M. Greenaway.
Vice-Presidents: F. Ray Hone, H. Maynard Rennie and E. H. Roche (New Zealand).
Censor-in-Chief: K. B. Noad.
Honorary Secretary: R. L. Harris.
Honorary Treasurer: Bruce Hall.

Councillors: Fellows: J. J. Billings, Professor C. R. B. Blackburn, M. E. Chinner, J. Eric Clarke, Professor Lorimer Dods, J. L. Frew, John Halliday, W. E. Henley, Bruce Hunt, C. G. McDonald, Sir William Morrow, O. Ellis Murphy, H. G. Wilson and Morvyn Williams. Members: J. C. English and T. H. Hurley.

Executive Committee: T. M. Greenaway,[1] H. Maynard Rennie, K. B. Noad, R. L. Harris,[1] Bruce Hall,[1] J. Eric Clarke, Sir William Morrow and O. Ellis Murphy.

Assistant to the Honorary Secretary: John R. Sands.

[1] *Ex officio.*

BOARDS OF CENSORS

Censor-in-Chief: K. B. Noad.

Australian Board: J. Mark Bonnin, J. Eric Clarke, J. L. Frew, S. J. M. Goulston, W. E. King and Sir William Morrow.

New Zealand Board: J. F. Landreth (Senior Censor), Professor J. E. Caughey, W. E. Henley, C. Graham Riley, E. H. Roche and Morvyn Williams.

COMMITTEES

Research Advisory Committee: The Research Advisory Committee has been reconstituted as follows: Professor Lorimer Dods (Chairman), Douglas Stuckey (Honorary Secretary), Professor C. R. B. Blackburn, Sir Macfarlane Burnet, Professor Sir John Eccles, Professor Sir Edward Ford, Bruce Hall,[1] R. L. Harris,[1] Bryan Hudson, C. G. McDonald and H. M. Whyte.

[1] *Ex officio.*

Therapeutics Assessment Committee: The following have been appointed to the Therapeutics Assessment Committee for the period 1961 to 1963: Professor R. R. H. Lovell (Chairman), M. V. Clarke (Honorary Secretary), Professor C. R. B. Blackburn, K. D. Fairley and Professor H. O. Lancaster.

Committee on Public Relations: The Committee on Public Relations is constituted as follows: Bruce Hall

(Chairman), the President, the Honorary Secretary, C. G. McDonald, K. B. Noad, G. L. McDonald, and John Sands (Public Relations Officer of the College). Public Relations Secretaries in the various States have been appointed as follows: C. B. Sangster (South Australia), Cyril Fortune (Western Australia), John Fitzwater (Queensland) and Bryan Hudson (Victoria).

Newsletter Editorial Committee: A Newsletter Editorial Committee has recently been drawn up to assist in the preparation of the College Newsletter. The Committee is constituted as follows: H. Maynard Rennie (Chairman), G. L. McDonald (Honorary Secretary), R. L. Harris,[1] Bruce Hall,[1] and Mr. A. L. Knight, with power to co-opt.

MEMBERSHIP

Admission of Fellows. The following Fellows were admitted on May 1, 1961, after election by the General Body of Fellows: *under Article* 44: Kevin Brennan, of Melbourne, and Professor W. H. Trethowan, of Sydney; *under Article* 42: P. J. Benjamin, W. J. G. Burke, E. D. Burnard, J. H. Deakin, M. L. Edwards, S. E. J. Robertson, T. I. Robertson and J. N. Sevier, of Sydney; A. J. M. Dobson, of Hobart; M. J. Etheridge and A. C. Schwieger, of Melbourne; John Fitzwater, of Brisbane; H. G. Rischbieth, of Adelaide; Christopher Gresson, of Christchurch; D. S. Malcolm, of Palmerston North; I. A. M. Prior, of Wellington; A. S. Turner, of Napier.

Admission of Members. The following candidates were successful at examinations held in Christchurch, New Zealand, on February 15, 1961: B. M. Barraclough, T. C. Highton, J. K. McKenzie and D. G. Palmer, of Dunedin; J. B. Blennerhassett and B. A. Scobie, of Wellington; B. M. Colls and R. N. Howie, of Auckland; E. A. Espiner and A. W. S. Ritchie, of Christchurch; J. B. W. Dunlop, of Palmerston North; R. P. G. Rothwell, of Hamilton; and P. B. Doak, of Papatoetoe. The following candidates were successful at examinations held in Sydney on April 19, 1961: W. Douglas and Ian Ferguson, of Brisbane; P. A. Castaldi, E. L. Corlette, R. W. Johnston, B. M. Learoyd, B. P. O'Connell, B. McK. Rush, R. R. Taylor and I. L. Thompson, of Sydney; J. P. Carew, J. A. Fuller, June L. Howqua, P. J. Kiernan, A. A. Large, C. R. Lucas, I. G. McDonald, Margery C. McKinnon, Aubrey Pitt, Bernard Sweet and J. R. York, of Melbourne; J. G. Mortimer, of Invercargill, New Zealand. The following candidates were successful at examinations held in Perth on April 27, 1961: John H. Allison, J. M. Angeloni, E. R. Beech, Michael Duffus, T. M. Gilbert, R. C. Godfrey, Paul Mestitz, W. G. Smith, J. T. Smyth and T. Waters, of Perth.

Honour. The honour of Officer of the Most Excellent Order of the British Empire has been bestowed by Her Majesty the Queen upon Dr. Cyril Fortune, of Perth.

Obituary. The Council records with regret the deaths of Professor Henry Priestley, of Sydney, a Foundation Fellow of the College; Dr. C. J. N. Leleu, of Fiji and Sydney; and Dr. E. H. Miles, of Sydney, who were members of the College; and Mr. M. C. Alder, of Sydney, Chairman of the Finance Advisory Committee of the College.

Membership. The College now has a roll of 11 Honorary Fellows, 378 Fellows and 665 Members.

GENERAL

Jubilee Fund Study Grants. Two Jubilee Fund Study Grants for the year 1961 have been awarded to Dr. Charles O. Crawford of Napier, New Zealand, and to Dr. David Nurse of Melbourne.

Pfizer Travelling Fellowship in Clinical Medicine (New Zealand). The Pfizer Travelling Fellowship in Clinical Medicine (New Zealand) for the year 1961 has been awarded to Dr. J. A. Kilpatrick of Dunedin.

Collier Travel Grant. A Collier Travel Grant has been awarded to Dr. Frank Croll of Sydney.

College Representatives. The following have been appointed to represent the College: Dr. J. Eric Clarke of Melbourne, on the Repatriation Central Medical Advisory Committee and on the Electoral College of Prince Henry's Hospital, Victoria; Dr. Kenneth Grice of Melbourne, on the Electoral College of St. Vincent's Hospital, Melbourne; Sir Alexander Murphy of Brisbane, on the Queensland Institute of Medical Research (reappointment); Dr. H. R. Gilmore of Adelaide, on the Postgraduate Committee of the University of Adelaide; Dr. C. B. Sangster of Adelaide, on the Advisory Committee of the Royal Adelaide Hospital; Dr. Cyril Fortune of Perth, on the Western Australian State Health Council; Dr. W. R. Pitney of Perth, on the National Heart Foundation; Dr. A. K. Cohen of Perth, on the Western Australian State Postgraduate Association; Sir Charles Burns of Wellington, on the Wellington Medical Research Foundation; Dr. W. E. Henley of Auckland, on the New Zealand Medical Research Council; and Dr. M. K. Gray of Christchurch, on the Canterbury Medical Research Foundation. The following have been appointed to the Advisory Boards of the Base Hospitals, Victoria: Dr. T. E. Lowe to the Ballarat and District Base Hospital; Dr. Keith Fairley to the Bendigo and Northern District Base Hospital; Dr. Robert Southby to the Geelong and District Hospital; Dr. J. Eric Clarke to the Gippsland Hospital (Sale); Dr. T. H. Steel to the Glenelg Base Hospital (Hamilton); Dr. Max Biggins to the Latrobe Valley Community Hospital (Yallourn); Dr. J. Horan to the Mildura Base Hospital; Dr. Theo Frank to the Mooroopna and District Base Hospital; Dr. M. V. Clarke to the Traralgon and District Base Hospital; Dr. L. Rothstadt to the Wangaratta and District Base Hospital; Dr. J. L. Frew to the Warrnambool and District Base Hospital; Dr. Stanley Williams to the West Gippsland Hospital (Warragul); and Dr. K. Grice to the Wimmera Base Hospital.

M. D. Silberberg Memorial Lecture. Council records with appreciation a donation of £1000 given by the Australian Fellowship of the Israel Medical Association to establish a lectureship to commemorate the late Dr. M. D. Silberberg, a past President of the Association and a Founder of the College. The Lecture will be

given biennially in rotation at State and Dominion Meetings of the College.

Gifts to Library. Gifts to the Library have been received with appreciation from Professor G. A. Ransome, Dr. J. R. Twiss, Dr. Edward Wilson, Sir Geoffrey and Lady Todd, Dr. Bryan Gandevia, Dr. T. H. Steel, Dr. Michael Kelly, Dr. Alex McGregor, Mrs. W. P. MacCallum, Dr. C. G. McDonald and Professor J. B. Cleland.

Future Meetings. The venue of future meetings of the College is as follows: 1961, the ordinary meeting will be held at Adelaide on October 11 to 13; 1962, the annual meeting will not be held, in view of the Australian Medical Congress to be held in Adelaide in May; the ordinary meeting will be held in Melbourne in August. 1963, the annual meeting will be held in Sydney and the ordinary meeting in Brisbane; 1964, the annual meeting will be held in New Zealand.

CORRIGENDUM

An error was made in the February issue of the Journal in the inclusion of the name of Dr. Bryan Gandevia, instead of that of Dr. Bryan Hudson, in the list of the subcommittee of the Victorian State Committee appointed to consider the Medical Salaries (Dillon) Report. Representatives of the Victorian State Committee who made submissions to the Medical Salaries (Dillon) Committee were Dr. Clive Fitts, Dr. J. J. Billings and Dr. Bryan Hudson.

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